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The Polarity of the Cell-wall of *Valonia*

BY

K. WILSON

(*Birkbeck College, London*)

With Plate V

ABSTRACT

Further evidence is presented to show that the systems of orientation of cellulose fibrils in the cell-walls of *Valonia* and *Dictyosphaeria* may be described by reference to two 'poles' upon which these orientation systems converge. The 'poles' are real structural features of the wall.

THE cell-wall of *Valonia* has long excited interest, and the main features of its multi-lamellate 'crossed-fibrillar' structure, first studied intensively by Preston and Astbury (1937), have become familiar. They showed that the striations visibly present in the wall represent lines of orientation of cellulose crystallites and they found that these lines fall into two systems, one forming a series of great-circle meridians about the cell and the other a series of spirals inclined obliquely to the meridians. The pattern of crystallites in the wall as a whole was thus described by reference to two 'poles', at which the meridians and spirals were visualized as converging.

In *Dictyosphaeria* a similar arrangement prevails, but in the smaller coenocytes of this plant the over-all picture is more readily appreciated; in particular the 'poles' are readily visible microscopically (Wilson, 1951). The principal difference from the account of *Valonia* given by Preston and Astbury is the conspicuous presence of a third striation direction,[1] so that in *Dictyosphaeria* there are, besides the meridians, two spiral systems, right- and left-handed. The 'poles' have constant positions in each coenocyte in relation to the plant as a whole, being located in the walls tangential to the surface of the thallus. In these walls the three systems of striations may be seen converging on the 'poles', whilst, in the anticlinal walls, of the three striation directions one is clearly meridional and the others are inclined to it at angles of about 120°. The meridional lines do show some curvature near the 'poles', so that they might, in a sense, be regarded as forming very steep spirals, but they differ so markedly in their form from the other obviously spiral systems that the distinction seems a valid one.

In a recent developmental study of the walls of *Valonia* spp. Steward and Muhlethaler (1953) have elucidated the initiation and early development of the wall in a striking manner. They find that the first-formed wall about a growing aplanospore has a random fibrillar structure; subsequently lamellae

[1] This occurs also in *Valonia*.

[Annals of Botany, N.S. Vol. XIX, No. 75, 1955.]

of the more familiar highly orientated type are laid down, and a regularly repeating sequence of three fibrillar directions is established: as the growing cell increases in surface area the outer, older lamellae of the wall become disrupted and new continuous lamellae are deposited internally. The aspects of this history of cell-wall development emphasized by them in discussion are those relating to the micro-structure and activity of the enzymic machinery responsible for the deposition of the wall: for reasons which they do not make entirely clear (since their own observations are not explicitly at variance with it) they reject the earlier evidence bearing upon the large-scale order of the fibrillar pattern about the cell as a whole (loc. cit., and also Steward and Muhlethaler, 1954). The reality of the 'poles' and the validity of the distinction between spiral and meridional fibrillar systems are called into question. In view of this, and the contrary opinions reiterated by Preston and Astbury (1954), these points merit further attention.

It is convenient here to refer firstly to *Dictyosphaeria*. The statement made previously that the 'poles' occur in the tangential walls of the cells is perhaps a trifle ambiguous in so far that the tangential wall at any point on a *Dictyosphaeria* thallus may consist of membranes of different cytoplasmic origin; the outer, somewhat torn, and often fragmentary, layers are the remnants of the walls of earlier cells within which daughter cells, each with its own wall, have arisen by segregative division. If, however, one refers to the wall properly belonging to each cell, then the 'poles' do occur in it as described. The polarization photomicrograph of a 'pole', previously figured (Wilson, loc. cit.), is of part of such a wall. This photograph is perhaps not entirely satisfactory in that some portions of the area represented by it, being in extinction positions, appear devoid of detail, and so introduce discontinuity in the visible pattern. The polarizing microscope was used, however, because it gives good contrast in the non-extinguished parts; moreover, rotation of the specimen allows detail to be seen in any part of it in a manner which cannot be portrayed in a still photograph, and in this way the courses of the striation systems can be followed. The reason for the use of the polarizing microscope was thus to display structure which is visible, though less easily, without the use of polarization phenomena. No detailed significance is attached to the extinction effects inevitably present, though the 'spherulite cross' of extinction (often somewhat distorted by local irregularities) does in fact draw attention to the mean over-all crystallographic radial symmetry peculiar to the 'polar' regions of the cell-wall. This, of course, arises from the combined effects of the superposed systems of crystallites converging in their three different ways on a common centre. The reality of the 'pole' may be further emphasized by reference to Pl. V, Fig. 1, which is a polarization photomicrograph of a single spiral lamella stripped from a 'pole'. The relationship of the spiral system to the 'pole', which is not strictly a point, but is a somewhat elongated, lenticular area, may be seen.

In the light of the microscopic evidence for the existence of the 'poles' in *Dictyosphaeria* it is interesting to turn to another optical property of these

regions of the wall. A tangential slice may be cut from a fragment of thallus so as to include either the inner or the outer walls of a number of cells, and if after being washed this is suspended in water and strongly illuminated obliquely from below in such a way that no direct light reaches the observer, a characteristic pattern of scattered light may be seen in each wall. This takes the form of a 6-rayed star (Pl. V, Fig. 2) and microscopic examination shows, as indeed one might suspect, that the centre of this star indicates the position of the 'pole'.

Without embarking on a rigorous explanation of this effect, one might liken it, rather loosely, to the familiar appearance of a gramophone record in oblique illumination. Here there is only one system of regular light scattering surfaces (the spiral grooves) and a 2-rayed scatter pattern results. In the *Dictyosphaeria* wall there are three such systems disposed about a common centre, the 'pole', and a 6-rayed pattern is thus produced.

If *Valonia* coenocytes, previously cut in half, washed out, and suspended in water, are similarly examined, the poles may be located in the same way (Pl. V, Figs. 3 and 4). A 2-lamellate strip, from a 'pole' found in this way, is shown under higher magnification in the polarization photomicrograph of Pl. V, Fig. 5, which may be compared with Pl. V, Fig. 1.

The light scatter pattern may be, as in *Dictyosphaeria*, 6-rayed (Pl. V, Fig. 3) or 4-rayed (Pl. V, Fig. 4). Even when it is 6-rayed, one pair of rays may be less evident than the other two pairs, suggesting that one system of striations is less well developed than the other two systems. In cells giving a 4-rayed pattern all three striation systems have, on closer examination, been found to be present, though one was weakly developed and was evidently insufficient to produce visible scattering. This weaker development of one system (which led Preston and Astbury to minimize its importance in their first study) may, as these authors suggest (1954), be a matter of the lamellae of this system being thinner or less crystalline than those of the other two systems, or alternatively the weaker system may be only irregularly represented in the repeating cycle of lamellar deposition. Though Preston and Astbury (1954) have emphasized the latter view, the dissection of macerated fragments of wall suggests that the former alternative is at least a contributory one, and there is some suggestion of differences in lamellar density in Pl. XVI (iv) of the account by Steward and Muhlethaler.

Irrespective, however, of whether there are two or three systems of fibrillar orientation in these cell-walls it seems clear that the 'poles' of these Valoniaceous coenocytes do exist, and that, in addition to those problems of wall deposition enumerated by Steward and Muhlethaler (1953, 1954), there is another problem, inherent in the structural symmetry of the cell *as a whole*, which can hardly be formulated in biochemical or biophysical terms, but which is none the less a real one.

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EXPLANATION OF PLATE

Illustrating K. Wilson's article on 'The Polarity of the Cell-wall of *Valonia*'

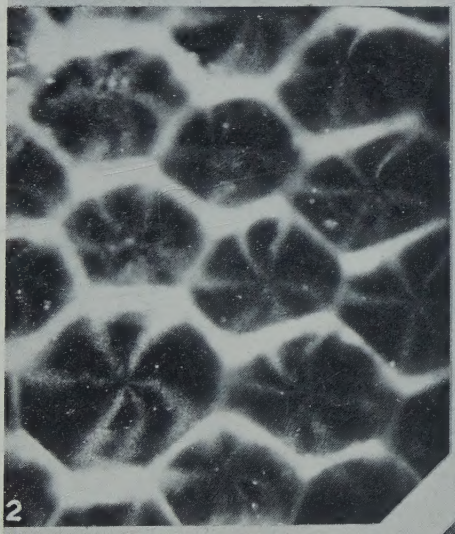
Fig. 1. *Dictyosphaeria favulosa*. A single lamella, belonging to one of the spiral systems, stripped from the region of a 'pole'. (Polarizing microscope, ×110.) The 'pole' appears as an elliptical area in the upper right-hand quadrant. Elsewhere the fibrillar pattern has been somewhat torn in stripping.

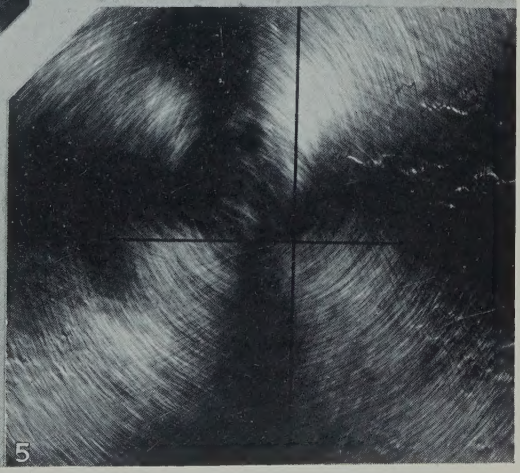
Fig. 2. *D. favulosa*. A thick surface section of a fragment of thallus, in oblique illumination, showing 6-rayed light-scatter patterns in the tangential walls of the cells. (×44.)

Fig. 3. *Valonia ventricosa*. A fragment of cell-wall, including a 'pole', in oblique illumination, showing a 6-rayed light-scatter pattern. One pair of rays is weaker than the other two pairs. (×6·5.)

Fig. 4. *V. macrophysa*. As Fig. 3, but showing a 4-rayed light-scatter pattern. (×5·5.)

Fig. 5. *V. ventricosa*. A 2-lamellate strip, from a 'pole'. (Polarizing microscope, ×90.) Compare Fig. 1.





K. WILSON

Fusarium Wilt of Bananas in Jamaica

I. Some Observations on the Epidemiology of the Disease

BY

J. RISHBETH

(*Plant Pathologist, W.I. Banana Research Scheme, Department of Agriculture, Jamaica*)[1]

With Plates VI and VII and eight Figures in the Text

Abstract

The banana wilt pathogen *Fusarium oxysporum f. cubense* can be detected in soil by a suitable host test. It often enters the host through living rootlets, from which it passes into the vascular strand of the main root and thence into the rhizome; apparently infection does not occur through dead roots.

The spread of wilt through plantations was studied by taking records at 2-monthly intervals: new cases arise both spontaneously and in association with pre-existing ones. Flooding is probably important in local dispersal of the pathogen, as it is in long-range dispersal. The relative importance of some other modes of dispersal is discussed.

The soil population of *F. oxysporum f. cubense* increases considerably when wilted bananas collapse and declines shortly after their removal. If the site is replanted with a banana variety resistant to wilt the pathogen can thereafter often be detected in the soil; in the absence of bananas, however, it cannot be detected by any test after about 10 years, although its continued survival is well established by many field observations on the incidence of banana wilt. Little is known about its mode of survival in soil.

Introduction

BANANA wilt, or Panama disease as it is often called, is still a major factor in banana cultivation. In the past it has caused havoc in most of the important banana-growing areas of the world and today it is increasing not only in newly established plantations, as in the Cameroons, but in older ones, as in East Africa, where until recently it was almost unknown. The Caribbean area has suffered as badly as any: in Jamaica, for instance, the approximate acreages abandoned through the disease in successive 6-year periods from 1914, soon after its appearance in the island, were 150, 2,200, and 13,300, an impressive indication of its rapid spread. The decline in banana cultivation has continued there until today the susceptible 'Gros Michel' variety is almost confined to scattered plantations in hill country. Since 1950, however, another variety, 'Lacatan', has been planted increasingly and this, although inferior to 'Gros Michel' in some other ways, is highly resistant to wilt. In the Caribbean area also much of the research on banana wilt has been carried out, and when the West Indian Banana Research Scheme was inaugurated

[1] Now at Botany School, University of Cambridge.

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in 1947 provision was made for continued investigations into the disease with a view to controlling it in 'Gros Michel'. In addition, great importance was attached to a programme for breeding new varieties resistant not only to wilt but also to Leaf Spot, caused by *Mycosphaerella musicola* Leach (Leach, 1946).

The work reported here was carried out in Jamaica during the 3 years 1950 to 1952. Field experiments to determine the effect of various soil treatments on the incidence of wilt occupied much of the research programme and considerable attention was given to further elucidation of factors affecting host resistance. During the course of the investigation a good deal of miscellaneous information was obtained from experiments and field observations; as was inevitable with a disease about which a good deal was already known, some aspects of the problem received much more attention than others. 'Gros Michel' proved very useful for experimental work and was used almost exclusively. The existence of a comprehensive review of the literature by Wardlaw (1935) makes it unnecessary to review here earlier work on this disease, concerning which relatively little has been published during the last 20 years. In this paper some observations are made on the epidemiology of banana wilt; the ecological status of the pathogen is also discussed and an account is given of its mode of dispersal and saprophytic phase. It is intended in subsequent papers to deal more fully with host-parasite relationships and to review attempted methods of control.

The Pathogen

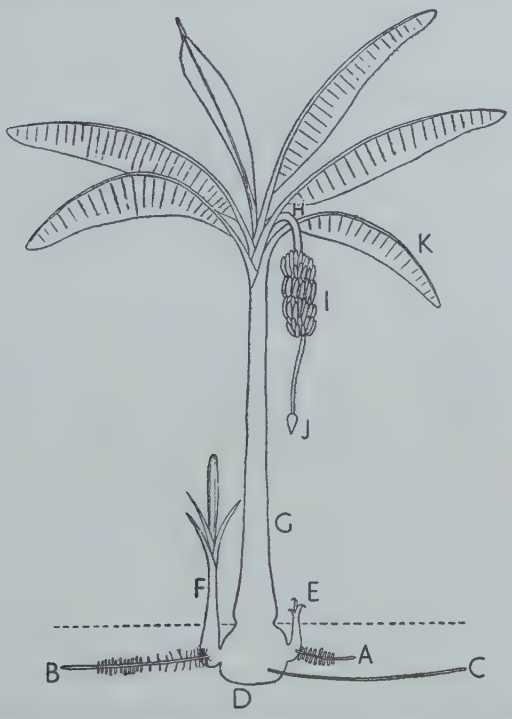
i. *Nomenclature*

In earlier stages of research into banana wilt there was a marked divergence of opinion about the possibility of recognizing the pathogen on the basis of morphology alone. Reinking and Manns (1933) held that this was possible and claimed that *Fusarium oxysporum f. 3*, whose distribution they recorded in Central American soils, was identical with the pathogen. Hansford (1926*a*) had earlier denied this possibility and provided evidence that a test for pathogenicity was essential. For reasons given later, the present investigation strongly supported Hansford's contention. In the present state of knowledge of *Fusaria* causing vascular wilt it seems desirable to follow Snyder and Hansen (1940) in regarding the banana pathogen as a biologic form of *F. oxysporum*: it will thus be designated *Fusarium oxysporum f. cubense* (E. F. Smith) Snyder and Hansen. All *Fusaria* of the section Elegans for which information about pathogenicity is lacking will be referred to the group species *Fusarium oxysporum* Schlecht. ex Fr. emend. Snyder and Hansen; where non-pathogenic status is established, specific reference to this will be made.

ii. *Occurrence*

Although nearly all previous investigators have recognized that banana wilt is a soil-borne disease, there has been a certain amount of confusion, arising as indicated above, about the occurrence of the pathogen in soil. Brandes

(1919) states that its presence in enormous numbers may be demonstrated by plating soil from banana plantations and adjoining fields although apparently he did not test the pathogenicity of his isolates. Reinking (1926) attempted to determine by inoculation experiments the relation of various *Fusarium* species to wilt and Colorado disease of bananas. Wilt was in fact obtained with only 7 isolates, all of which originated from 'Gros Michel', 5 being from vascular



TEXT-FIG. 1. Diagram illustrating the structure of a mature 'Gros Michel' banana. A, young root with turgid rootlets; B, older root with some withered rootlets; C, long mature root with no rootlets remaining at proximal end; D, rhizome; E, young bud or 'sucker'; F, older bud with small shoot; G, pseudostem, consisting of overlapping leaf-bases; H, flowering stem which during development grew up through the pseudostem; I, fruit bunch; J, male inflorescence; K, leaf.

bundles of wilted plants, 1 from the exterior of the cut surface of an infected pseudostem, and 1, rather surprisingly, from dead floral parts at the end of a fruit (the structure of a mature banana plant is shown diagrammatically in Text-fig. 1). Of his isolates from soil, which included several closely resembling the banana pathogen, none proved pathogenic. Reinking and Manns nevertheless quote this work in support of their contention that *F. oxysporum f. 3*, isolated from soil, is identical with the banana pathogen. They state that this fungus could only be isolated from soil around diseased banana plants and could not be isolated from soil around healthy ones or from soils which had not previously borne bananas.

Hansford (1926*a*), working in Jamaica, found that isolates of Elegans *Fusaria* from vascular bundles of wilted bananas, varying considerably in morphology, produced typical symptoms of the disease in 'Gros Michel'. By contrast, isolates of very similar appearance obtained from soil, dead banana leaves, and other plant debris were invariably non-pathogenic. Hansford deduced that the pathogen is absent from soils where bananas are not attacked by the disease. Ward (1930) found in Malaya that a species of *Fusarium* morphologically similar to the banana pathogen was present both in cultivated and virgin soil, but susceptible varieties of banana planted in the latter were not readily attacked by the disease.

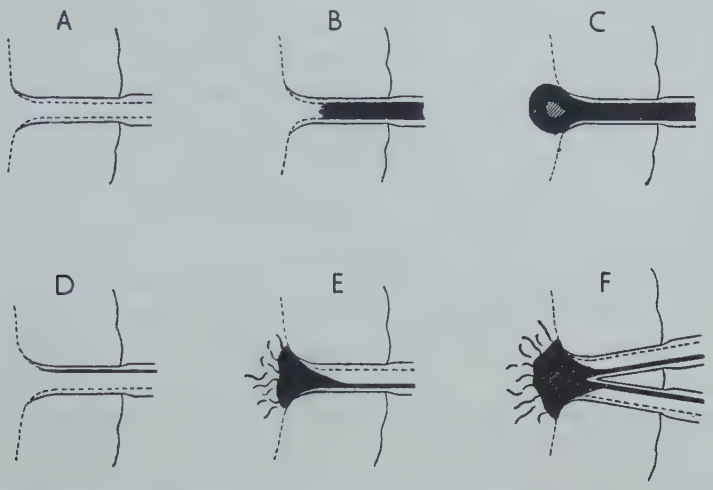
In view of the important bearing of such observations on the status of the pathogen, it was decided in the present investigation to test the pathogenicity of a range of *F. oxysporum* isolates. Isolates were obtained from the vascular tissue of bananas by plating on to 2 per cent. malt agar and from soil by the soil-plate method of Warcup (1950), using Dox's agar. Where necessary, cultures were freed from bacteria by transferring to acidified potato dextrose agar. From each isolate a single-spore culture was obtained and this was kept either in sterile soil or on a potato dextrose slope in a screw-capped tube. Cultures maintained by the latter method dried out very slowly at the storage temperature of 28–30° C., sub-culturing being necessary only at about yearly intervals. The morphology of the various isolates was not studied in detail, but it appeared, as Hansford maintained, that the range of morphological types occurring in diseased bananas was paralleled by the range existing in soil: no clear distinction between the two was possible.

Earlier investigators tested pathogenicity of *Fusarium* isolates by growing a susceptible banana variety in large containers of sterilized soil into which a bulky inoculum was introduced. Wilt commonly developed within 4–8 months if the isolate used was pathogenic. This test has the great merit of giving easily recognized symptoms with pathogenic isolates but is unduly cumbersome where large numbers of isolates are to be tested or where considerable replication is desired. Nor are the results given especially consistent, judging by the experience of Reinking (1926). Of isolates tested by him more than once, 2 produced wilt in each of two tests, 1 produced wilt in one out of two tests, and 1 in only one out of five tests.

In the present investigation, glasshouse experiments were carried out with 'Gros Michel' suckers, of girth 13–17 in., grown in 12 in. porous earthenware pots. It was thought at first that infection might be achieved most rapidly by direct inoculation of the fungus into the roots or rhizome, but this method in fact proved ineffective. During these preliminary trials it was discovered by chance that with some isolates small amounts of inoculum introduced into the soil caused infection through rootlets (Pl. VI) and that, after the fungus reached a main root, it grew in the vascular tissue and eventually entered the rhizome. Allowing for the small size of sucker, such rhizome infections closely resembled early stages of the disease found in the field. It was further discovered that other isolates, for instance from soil, would not

produce such infection and hence a basis for testing pathogenicity was established.

In the method finally adopted plants were grown in unsterilized soil, a light alkaline loam from a site which had never borne bananas. Banana suckers, obtained from a disease-free site, were trimmed and thoroughly washed before planting. Pots were watered every day for the first 5 weeks



TEXT-FIG. 2. Diagrams of banana root bases cut longitudinally to show various conditions found in inoculation experiments with plants grown in pots of unsterilized soil (× 1). The position of vascular tissue in the root base and rhizome is indicated by dotted lines.

Diagram.	Condition.	Score for count of rhizome infection.
A	Healthy	0
B	Vascular infection sealed off at root base, usually dark red in colour	0
C	Limited 'pocket' rot of rhizome caused by *Cylindrocarpon* sp.; no progressive vascular infection. Vascular strand of root usually yellow; rot dull reddish-purple	0
D	Vascular infection probably about to enter rhizome, pale yellow to orange	0
E	Vascular infection, caused by *Fusarium oxysporum f. cubense*, shortly after entering rhizome, orange-yellow, later dark brown or purple	1
F	As last, but rhizome infected almost simultaneously from two roots	2

and thereafter on alternate days. Since pots were raised on small wooden supports about an inch above the concrete floor of the glasshouse and were virtually unshaded, considerable fluctuations of soil moisture content occurred and this almost certainly promoted root infection. Plants were inoculated 2–4 weeks after planting, the longer interval being desirable where growth for any reason was slow. The inocula employed were *F. oxysporum* cultures growing on a mixture of fine sawdust (85 per cent.) and maize-meal (15 per cent.), initially brought to a moisture content of about 120 per cent. Two

grammes of air-dried culture, commonly having a total count of some 10^8 viable spores, was mixed with the surface soil, care being taken to cause minimal damage to roots. Plants were taken up after 3 months when the number of rhizome infections was counted by cutting the suckers radially into slices about 1 cm. thick and examining each root base. Text-fig. 2 illustrates some of the conditions found at root bases; their assessment for purposes of the count is also indicated. For each isolate three 'Gros Michel' suckers were inoculated and of these two were examined at the end of the experiment, the third being available in the event of growth failure or doubt about the pathogenicity of the isolate. About twenty isolates were tested at a time and as a safeguard each batch also contained a set inoculated with a known pathogenic isolate, a set inoculated with a known non-pathogenic isolate, and a third uninoculated set. Differences were usually clear cut: in three batches, for instance, pathogenic isolates gave an average of 8·1 infections per rhizome and non-pathogenic ones 0·2 infections per rhizome. Over the period of testing, uninoculated controls had on the average 0·1 infections per rhizome. The method gave reasonably consistent results since in many separate trials a pathogenic isolate produced rhizome infections in 19 out of 20 plants and a non-pathogenic one failed to produce any infection in 12 plants.

It should be explained that small 'Gros Michel' suckers grown under these conditions behaved differently from larger suckers planted in the field in certain important respects. Roots were readily infected even when the inoculum was very small and, by the end of 3 months, the pathogen had grown sufficiently to enter the rhizome from some 90 per cent. of roots infected through rootlets. This indicates that resistance to infection was very low and, incidentally, that roots having a characteristic vascular discoloration could fairly safely be assumed to contain the pathogen. On the other hand, at first sight in contradiction to the above, the fungus seldom progressed far in the rhizome, rarely entered the pseudostem and hardly ever caused wilting. The possible reasons for this will be considered elsewhere. Since host resistance was low, any *F. oxysporum* isolate failing to show pathogenicity in this pot test would be most unlikely to behave as a wilt pathogen in the field; the test might, however, over-estimate parasitic ability. For this reason it seemed desirable to carry out further tests with apparently pathogenic isolates under field conditions.

Suckers were planted in a small plot which had not previously borne bananas, and the soil around each plant was heavily inoculated with a sawdust/maize-meal culture about 3 weeks later. Sixteen *F. oxysporum* isolates were tested, of which, on the basis of the pot test, 8 had been classed pathogenic and 8 non-pathogenic. For each isolate 2 plants were inoculated, 4 pairs of uninoculated plants serving as controls. Nine months after planting results were as follows: 7 of the 8 presumed pathogenic isolates had each produced one case of wilt, none of the 8 presumed non-pathogenic isolates had produced wilt, but 1 control plant out of 8 was affected. Shortly afterwards other controls became affected and results were confused, probably because close plant-

ing (8 ft.×8 ft.), dictated by the small size of plot, allowed cross-infections. This experiment, though unfortunately rather small, suggests that results obtained from the pot test are reliable and that pathogenic isolates may fairly confidently be classed as *F. oxysporum f. cubense*. Isolates classed as non-pathogenic may well be of varied status: they doubtless include soil saprophytes some of which cause limited root infection of bananas under certain conditions.

The results obtained with isolates from different sources were striking. Of isolates from the rhizome or pseudostem of plants showing typical wilt, 53 out of 58 (91 per cent.) proved pathogenic; of the other 5, some at least had probably lost pathogenicity during prolonged culture. Thirty-three of these isolates were from Jamaica, 22 from Trinidad, and 3 from East Africa. Just over half were from 'Gros Michel', the remainder being from other banana varieties, including new tetraploids: thus isolates from a wide range of varieties are also pathogenic to 'Gros Michel'. Only 11 out of 38 (29 per cent.) isolates from variously affected roots were pathogenic, although the proportion of pathogenic isolates was greater (7 out of 8) from roots with vascular discoloration characteristic of early stages of infection. By contrast, not 1 of 50 isolates from soil was pathogenic despite the fact that a third of the soils from which they were obtained bore diseased plants. This shows that it is exceedingly difficult to detect *F. oxysporum f. cubense* in soil by plating, and hence that its soil population cannot be estimated in this way.

In view of this, attempts were made to detect the pathogen in soils by means of another pot test. It is more convenient to describe this later, but some of the results obtained may be anticipated here. *F. oxysporum f. cubense* was detected in the soil of many banana plantations, most but not all of which were affected by wilt, and it was also detected in soils up to about 8 years after the removal of diseased bananas. It was not detected in soils which had never borne bananas.

So far, then, it appears that the banana pathogen is indeed a soil-borne organism, but not one so abundant or so readily isolated from soil as was earlier imagined. It cannot be differentiated from morphologically similar *Fusaria* except on the basis of pathogenicity. Its presence in soils of banana plantations is not invariably correlated with that of wilt and, as one might expect, it is scarce or apparently absent in soils where bananas are not growing. The difficult question of its occurrence in soils which have never borne bananas is bound up to some extent with host range and will therefore be discussed below.

iii. *Host range*

The various parasitic forms of *F. oxysporum* are characterized by a limited host-range. This is well illustrated by *F. oxysporum f. conglutinans* (Wollenw.) Snyder and Hansen, the various physiologic races of which will infect members of the cabbage tribe and some other Cruciferae. Armstrong and Armstrong (1952) report that isolates from cabbage and radish failed to produce

wilt in wilt-susceptible plants of 18 genera not in Cruciferae, and that the same number of different wilt *Fusaria* from various hosts did not cause wilt in one variety each of cabbage and radish. The latter result corresponds to that obtained with the banana. One or more isolates of *F. oxysporum* from the following hosts were tested for pathogenicity to 'Gros Michel' in the manner already described:

Cabbage	*F. oxysporum f. conglutinans* (Wollenw.) Snyder and Hansen
Brussels sprout	,, ,, ,, ,,
Pea	,, *f. pisi* (Linford), race 1, Snyder and Hansen
,,	,, *f. pisi* Snyder, race 2, Snyder and Hansen
Tomato	,, *f. lycopersici* (Sacc.) Snyder and Hansen
Sweet potato	,, *f. batatas* (Wollenw.) Snyder and Hansen
Cotton	,, *f. vasinfectum* (Atk.) Snyder and Hansen
Oil palm	*F. oxysporum* Schlect. ex. Fr.
Sugar-cane	,, ,, ,,

In no instance was rhizome infection obtained.

F. oxysporum f. cubense is recorded most frequently from edible bananas, *Musa sapientum* L. (sensu lato) and *M. paradisiaca* L., but it causes wilt in some other *Musa* spp. such as Manila hemp, *M. textilis* Née. Experimental evidence about its further possible host range is scanty. Waite and Dunlap (1953), in a preliminary investigation, claim that three species of grass and *Commelina diffusa* N. L. Burm. serve as alternate hosts for *F. oxysporum f. cubense* on the grounds firstly that the fungus colonized roots of such plants grown in sterilized soil which had been liberally inoculated, and secondly that isolates morphologically indistinguishable from the pathogen were obtained from roots of these species growing in naturally infected soils. Whilst it seems reasonable that the pathogen colonizes roots of some species when its soil population is artificially high, proof that it will do so to a significant extent under natural conditions, when its soil population is much lower, must depend on adequate demonstration of the pathogenicity of *Fusarium* isolates from such roots.

Brandes (1919) reports that cotton seedlings grown in sterile soil heavily inoculated with *F. oxysporum f. cubense* were stunted and that there was some root injury. In the current investigation, cotton seedlings were grown under similar conditions, but the inoculum, although adequate to infect 'Gros Michel', was not as heavy as that used by Brandes and no infection resulted. Brandes rightly did not claim from his results that the banana and cotton wilt pathogens were identical. A relevant observation is that of Houston and Knowles (1953) who worked with *F. oxysporum f. lini* Snyder and Hansen. They found that many flax plants of nearly any variety, regardless of the previously recorded degree of resistance, could actually be killed by pre-emergence or seedling infection if a heavy inoculum was used and environmental conditions were optimum for the fungus. It seems reasonably certain that with adult plants the various parasitic forms of *F. oxysporum* attack a limited range of closely allied hosts, such as certain varieties of a species. With seedlings, however, the relationship is far less specialized and plants

distantly related to the true hosts may sometimes be attacked. It is thus dangerous to try to establish the host range of parasitic forms of *F. oxysporum*, or indeed to estimate the pathogenicity of *F. oxysporum* isolates for a given host, solely by inoculation tests with young seedlings.

Brandes inoculated plants of genera related to *Musa*, such as *Ravenala*, *Heliconia*, and *Strelitzia*, and obtained no infection, though he does not state whether he examined roots or whether he used absence of wilt as the sole criterion. Though it seemed unlikely that *F. oxysporum f. cubense* could cause wilt in any wild species related to the banana, it was thought desirable to investigate further the effect of the pathogen on the roots of such a species. The genus *Heliconia* was chosen for this purpose in view of its wide distribution in the Caribbean area. Small plants of *H. psittacorum* L. (native in forests of West Indies and South America) were planted in pots of light loam and watered on alternate days. Pots were divided into three series, one of which received a weekly application of ammonium sulphate (1 g. in 100 ml. per pot) from the fourth week onwards. After 6 weeks a sawdust/maize-meal culture of *F. oxysporum f. cubense*, adequate to infect 'Gros Michel', was added to the surface soil of pots receiving nitrogen and to those of one other series; the third series received sterile sawdust/maize-meal. Roots and rhizomes were examined after a further 8 weeks. In the control series the proportion of healthy roots was 60 per cent., whereas in the inoculated but otherwise untreated series it was 23 per cent. and in the inoculated series receiving nitrogen it was only 3 per cent. There were no rhizome infections in 6 plants of each of the first two series, whereas in the third one 4 out of 10 plants had limited rhizome infections (Pl. VI, A). Affected roots showed various conditions ranging from slight vascular discoloration to extensive die-back, which was most pronounced in the series receiving nitrogen (Pl. VI, B). *F. oxysporum* was readily recovered from roots and infected rhizomes, and an isolate proved pathogenic to 'Gros Michel'. The effect of nitrogenous fertilizers in lowering the resistance of roots and rhizomes to invasion by the pathogen was already well known from pot experiments with 'Gros Michel': with *Heliconia* such fertilizing helped to demonstrate that some at least of the root rot was caused by the wilt pathogen.

Although this experiment is open to the criticism that the plants, being small and growing under artificial conditions, were probably less resistant than plants growing under natural conditions, it seems likely that *F. oxysporum f. cubense* could cause a limited root infection of wild plants and hence could be maintained to some extent in natural communities, as occurs with resistant banana varieties in plantations (p. 321). If this were the case, it might further be postulated that the pathogen is indigenous in forests containing *Heliconia* and perhaps in those containing other genera related to *Musa*.

Several observations suggest that *F. oxysporum f. cubense* may in fact be indigenous in the Caribbean area. On one occasion a portion of a heavy forest in Central America, remote from habitation, was planted with disease-free suckers and within 2 years no less than 75 per cent. of the bananas

developed wilt. Here, probably, the pathogen was already present in the soil: it could hardly have arisen within such a short period by mutation, for instance. Wardlaw (1941), referring more generally to the incidence of banana wilt in Central America, states that conditions on the floor of virgin forests (where *Heliconia* spp. are often present) appear to be specially favourable for the propagation and distribution of the pathogen. Smith (1932) states that numerous observations made in mountainous districts of the parish of Portland, Jamaica, suggest that *F. oxysporum f. cubense* is indigenous there. In Jamaica, *Heliconia* spp. are characteristic components of the shrub layer of lower montane rain forest which is now very limited in extent owing to clearing (Asprey and Robbins, 1953). It may well be significant that one of the main relict areas of this forest type is located at the head waters of the Rio Grande, the very area in which Smith thought *F. oxysporum f. cubense* to be indigenous. By contrast, there is no evidence to suggest that extensive rain forest ever occurred in the Jamaican lowlands, which might explain the initial absence of the pathogen from plantations on alluvium.

It would be unwise, however, to assert that all outbreaks of wilt in Jamaica were ultimately attributable to dispersal of an indigenous pathogen, although this might conceivably have happened. Before wilt appeared in the island, not only had slightly susceptible varieties such as the 'apple' banana been cultivated, perhaps for centuries, but 'Gros Michel' itself had been grown for 75 years and had been an important export crop for over 30 years. During this long period of banana cultivation the pathogen might have arisen by mutation from a saprophytic strain of *F. oxysporum* and have multiplied by infecting banana roots. The scattered and almost simultaneous outbreaks of wilt which occurred from 1911 onwards could be explained by a previous gradual build-up of the pathogen and, perhaps more importantly, by a progressive deterioration of soil conditions through banana cultivation (this point will be elaborated in a further paper). It was established beyond doubt in the present investigation that *F. oxysporum f. cubense* occurs in the soil of certain 'Gros Michel' plantations where crown symptoms of the disease are entirely lacking: hence the pathogen could in the past have been fairly widely dispersed before its presence was suspected. It seems on the whole less likely that these initial outbreaks arose from separate introduction of the pathogen from abroad, as has been suggested.

Banana Wilt

i. *Early stages of infection*

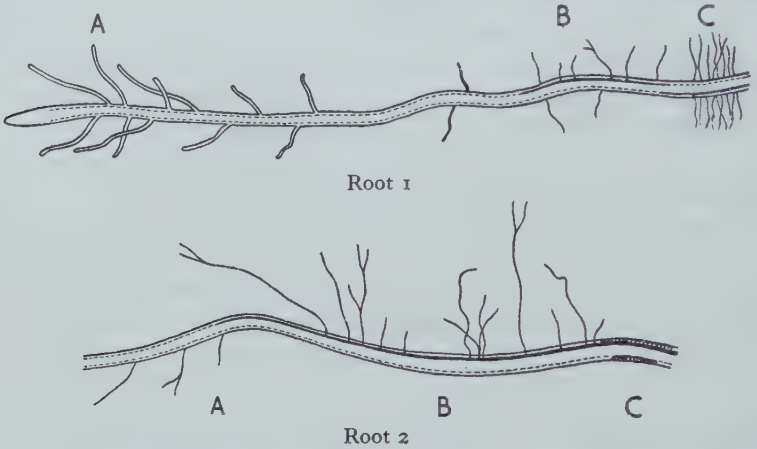
Previous workers have all agreed that infection frequently results from the use of banana suckers already infected from the parent rhizome. There has also been general agreement that the aerial parts of bananas cannot be successfully inoculated with *F. oxysporum f. cubense* and are most unlikely ever to serve as infection courts. The precise mode of entry of the pathogen from the soil has been disputed, however. Drost (1912), on the basis of field

observations, believed that initial root infection occurs through minute holes left by the death and subsequent decay of rootlets. Brandes (1919), basing his conclusions on direct inoculation of washed roots and rhizomes, believed that the fungus enters through wounded rhizomes and young unwounded roots, but not through rootlets. The pathogen was believed to penetrate the epidermis of young fleshy roots and to grow through the cortex to the vascular tract, where it then grew more rapidly than elsewhere. Wardlaw (1930*a*, 1930*b*, 1931), on the other hand, showed that direct infection of the rhizome is prevented by the formation of suberized cambiform barriers and is unlikely to occur under natural conditions. He further showed conclusively that critical rhizome infections occur through diseased root bases. Wardlaw studied early stages of infection by inoculating 'Gros Michel' roots in moist chambers and demonstrated that under these conditions actively growing roots did not become infected; he also showed that an infection established at the distal end of a rootlet gradually passes back into the cortex of the parent root, where again progress of the pathogen may be restricted by structural changes. In pot experiments Wardlaw obtained root infection from soil only where roots were subject to fluctuating moisture content, all stages of direct infection, from shallow to deep invasion of the cortex, being observed. Severe root infections occurred when healthy roots were plasmolysed for a short time in the presence of the pathogen. Root disease was thought to be chiefly important when it takes place close to the root base; infection may then pass into the rhizome.

Some at least of these divergent observations might well be attributed to the use of exposed roots and very heavy inocula; and in the field complications undoubtedly arise from the difficulty of discriminating between root symptoms caused by the pathogen and those caused by other strains of *F. oxysporum*.

In the present investigation, stages of root infection were observed both in glasshouse pot tests and in plantations. In the former, similar findings were made with a variety of soil types, naturally or artificially infected. Conditions differed from some of the experiments set up by Wardlaw in that pots did not stand in water and in general there was probably a greater fluctuation in soil moisture content. It should first be mentioned that banana roots growing out from a rhizome usually develop at once a dense cluster of rootlets and during further growth tend to bear scattered rootlets only (Text-fig. 3, root 1). Rootlets are at first white and turgid, but they become discoloured after about 2–3 weeks, and then soon wither and blacken: all stages in this process may be seen along a single root. The fungus never directly invaded intact main roots at any stage of development but it frequently invaded mature rootlets. The precise mode of entry was not determined microscopically, but in the earliest stage of infection observed the pathogen had entered the distal point of the rootlet and only this region was discoloured. There was no suggestion that entry occurred after rootlets had died. By the time the fungus had reached the base of a rootlet it was already well established in the vascular strand and might thus pass directly into that of the main root. A characteristic

red or yellow discoloration, corresponding with further growth of the fungus, soon extended along the main root from the initial point of entry, developing more rapidly towards the rhizome than distally (Pl. VI). By the time the fungus had reached the rhizome the main root, which until then had appeared outwardly healthy, often developed small areas of cortical rot (Text-fig. 3, root 2): at first the epidermis was intact and the cortex discoloured brown,



TEXT-FIG. 3. Drawings of banana roots from young plants growing in pots of inoculated soil, detached at the point of origin from the rhizome: two half-roots ($\times \frac{1}{2}$ and $\times 1$) showing early stages of infection with *Fusarium oxysporum f. cubense*. The position of the vascular strand is indicated by dotted lines.

Region.	Extent (cm.).	Characteristics of region.
Root 1:		
A	10	Rapidly growing apical region with white, turgid rootlets, clustered near apex; healthy.
B	7	Central region with scattered, withered rootlets; limited vascular infection arising from four rootlets.
C	3	Proximal region with densely clustered, withered rootlets; vascular infection arising from one rootlet and entering rhizome.
Root 2:		
A	3	Cortex healthy, no infection through rootlets; vascular strand infected from region B, its discoloured zone narrow and yellow to orange in colour.
B	4	Cortex healthy; much infection through rootlets; vascular strand with a wide discoloured zone, very dark red in colour.
C	1	Cortex with blackish lesions (indicated by dots); vascular infection passing into rhizome.

but later the epidermis died and the lesion became black. Occasionally such lesions developed at the site of initial infection, but more often they were located near the root base and were thus secondary to vascular infection of the main root. Complete die-back of roots was infrequent except with heavy inocula.

Most infections occurred in the region of scattered rootlets between 5 and 15 cm. from the rhizome, well below the soil surface, and usually at a distance from the inner pot surface. With growth in pots, a solitary rootlet infection

some distance along a main root could eventually lead to penetration of the rhizome. Where many rootlet infections occurred, the proportion of discoloured vessels in the vascular strand of the main root was correspondingly larger. When unrooted suckers were planted in infected soil the rate of root production largely determined how soon initial infection occurred. In summer, root infections were found within 4 weeks of planting, rhizome infections occurring about a week later, whereas in winter, root infection was often delayed until some 8–9 weeks after planting. In any one batch of plants there were often considerable differences in the rate of rooting and hence in the rate of development of infection. If plants with mature roots were transferred to infected soil, root infections occurred within a week.

Further evidence about root infection was obtained from a pot experiment in which banana suckers were grown in an unsterilized light loam inoculated with the pathogen. Three weeks after planting the young root systems were

TABLE I

Results obtained by growing Young Banana Plants in pots of Unsterilized Soil inoculated with Fusarium oxysporum f. cubense: *the Effect on Root and Rhizome Infection of damaging the Roots*

	Percentage roots in various conditions.					
		Infected from				
Root treatment.	Healthy.	Rootlets.	Dead tip.	Cortical rot.	Dead.	Number of infections per rhizome
None . . .	72	25	0	0	3	2·1
Damaged by transplanting . .	64	20	5	0	11	2·3
Damaged by plasmolysis . .	60	8	0	8	24	1·1

damaged by taking up and replanting or by flooding the soil with a 2 per cent. solution of sodium chloride, the latter treatment being similar to that used by Wardlaw (1931), though he used a different type of soil. Each treatment was carried out with 10 plants, one control set of 10 being left untreated. After a further period of 6 weeks, plants were taken up and all roots except very young ones were examined; in addition rhizome infections were counted. Attempts were made to discover the mode of initial entry into roots, though this was uncertain when the root had died back or had suffered severe cortical rot and was of course impossible to determine when the root had died. The results, obtained from examination of between 60 and 90 roots from each set, are given in Table I.

Inoculated but otherwise untreated plants had a high proportion of healthy roots and all other living roots were infected through rootlets. The replanted series suffered slight root damage, judging by the increased proportion of dead roots, but this did not appreciably affect the number of rhizome infections. By contrast the series treated with sodium chloride suffered severe root injury,

the great majority of roots having visible lesions, and this was correlated with the death of many roots, the appearance of vascular infection associated with cortical rot, and a low incidence of rootlet infection. Rhizome infection was reduced, however. In another experiment, suckers were transferred to inoculated soil after growing for 3 weeks in sand. Here, with plants not further treated, as many as 55 per cent. roots were infected through rootlets, and rhizome infections averaged 3·3 per plant. With comparable plants treated with sodium chloride, only 19 per cent. roots showed rootlet infection and twice as many uninfected roots were present; rhizome infections averaged only 1·9 per plant. Under the particular conditions of these experiments, therefore, intact or slightly damaged roots were chiefly infected through rootlets, whereas roots heavily damaged by plasmolysis were to some extent infected in this way and possibly also in other ways, although study of earlier stages would be required to show this. Severe root damage, far from increasing rhizome infection, actually depressed it.

In the foregoing experiments the pathogen had entered the rhizome through relatively few roots which were dead at the time of examination, and it was not clear whether invasion of such roots had occurred before or after their death. To investigate the ability of *F. oxysporum f. cubense* to invade dead roots, more suckers were rooted in sand. After 6 weeks the suckers were taken up and with each about one-third of the roots were scalded by immersion for $\frac{1}{2}$ minute in water at 90° C.; roots thus treated were clearly marked and the plants then transferred to inoculated soil. Five weeks later it was found that none of a total of 12 scalded roots (which had evidently died soon after treatment) were associated with rhizome infections whereas 19 out of a total of 24 (79 per cent.) comparable untreated roots had been infected sufficiently to produce rhizome infections. This result agrees with the general observation, mentioned earlier, that rootlets are not colonized by the pathogen after they are dead. Although the possibility that *F. oxysporum f. cubense* colonizes dead banana roots to some extent is not ruled out, it seems very doubtful whether this pathogen ever enters a rhizome through dead roots under natural conditions, where the soil population of the fungus is lower than in this experiment.

It is suggested that the results obtained in this short series of experiments may be explained in terms of the selective advantage held by *F. oxysporum f. cubense* over other soil fungi, whereby with healthy roots a proportion of mature rootlets are invaded, leading in turn to root and rhizome infection. With dead roots, by contrast, the advantage tends to lie with other more numerous soil fungi, many of which grow faster than the pathogen. With roots damaged by plasmolysis the condition is intermediate: large numbers of rootlets are presumably killed outright and the number of infection courts is correspondingly reduced. In a similar experiment Wardlaw (1931) obtained no root infection with inoculated but otherwise untreated plants and uniform infection with comparable plants whose roots had been subjected to plasmolysis. This result was probably connected with the type of soil, potting

compost, used by Wardlaw: the moisture content would have been higher and more uniform than with the light loam used in the current experiments, and this may well have been sufficient to inhibit root infection in control plants. Damage by plasmolysis might then have lowered the resistance of roots sufficiently to allow infection. The experiments described throw little light on the hypothesis of Keyworth and Dimond (1952) that resistance of tomatoes to infection by *F. oxysporum f. lycopersici* is increased through a chemical response to wounding. This is not altogether surprising since the plants concerned are so different anatomically; but it is perhaps worth noting that with the banana there is no evidence that infection of a rhizome through uninjured roots is inhibited by extensive damage to other roots. Here, extent of infection is more probably related to availability of infection courts.

TABLE II

The Incidence of Fusarium *Species in Various Types of Naturally Infected Banana Root, as shown by Plating, and the Pathogenicity of some* F. oxysporum *Isolates obtained*

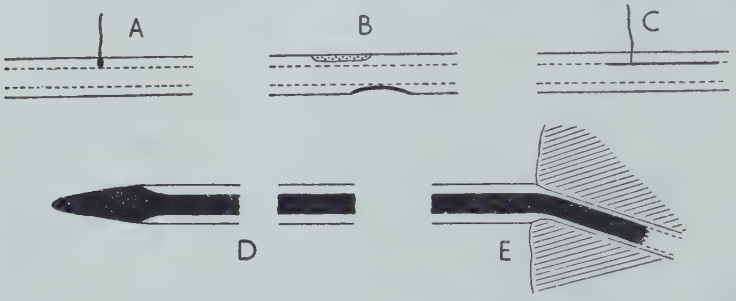
Type of infection (as in Text-fig. 4).	Total No. of samples examined.	No. of samples from which there were isolated:			Pathogenicity of *F. oxysporum* isolates.	
		Fusarium oxysporum.	(% with *F. oxysporum.*)	Other *Fusarium* spp.	No. tested.	No. pathogenic.
A	29	1	(3)	0	2	0
B	30	1	(3)	2	5	0
C	50	34	(68)	0	8	7
D	42	11	(26)	4	5	1
E	72	15	(21)	32	12	1

In connexion with the view of Brandes (1919) that *F. oxysporum f. cubense* causes infection through the cut end of rhizomes, it may be recorded that, in pot experiments with unsterilized soil, tissues in this region regularly became infected by a strain of *F. oxysporum*. This caused a deep red discoloration somewhat resembling that found in rhizomes infected by the pathogen. Penetration was usually restricted to a depth of about 1 cm. but was sometimes deeper. Pathogenicity tests with several isolates showed that the strain was incapable of entering rhizomes through roots and was therefore not *F. oxysporum f. cubense*.

Observation of root infection in the field is complicated in several ways. It is not easy to discover early stages of natural infection since wilt only occurs when a rhizome is fairly heavily infected, by which time the root or roots through which the pathogen entered are extensively rotted. Further, vascular discoloration in roots is not always a safe guide to the presence of the pathogen, by contrast with pot experiments. Information was obtained about the distribution of *F. oxysporum* in banana roots taken from several plantations. Table II gives the number of root samples of various kinds from which this and other species of *Fusarium* were isolated by plating and also records, for

the limited number tested, the pathogenicity (i.e. ability to infect rhizomes) of the *F. oxysporum* isolates. The various types of infected root from which samples were obtained are illustrated diagrammatically in Text-fig. 4.

F. oxysporum was rare in sealed-off vascular strands of rootlets which had produced no discoloration in the main root and the single isolate tested was non-pathogenic. It was similarly rare in all stages of cortical rot of the main root. To obtain further isolates from this source for a pathogenicity test, cortical lesions were incubated in moist Petri dish chambers: of five isolates thus obtained, none proved pathogenic. The scarcity of *F. oxysporum* even in young cortical lesions suggests that the pathogen rarely enters roots in this way. Cortical rot must generally arise independently of the pathogen, from



Text-fig. 4. Diagrams showing portions of banana root cut longitudinally to illustrate various types of infection occurring naturally (× ⅔). The position of the vascular strand is indicated by dotted lines. A, infection from a dead rootlet sealed off at point of entry into vascular strand of main root; B, cortex of main root with various stages of rot; C, vascular discoloration of main root: early stage associated with infection through a rootlet; D, vascular discoloration of main root: later stages associated with die-back or of unknown origin; E, vascular discoloration of main root: infection sealed off at root base.

a variety of causes; occasionally, however, it is likely to follow vascular infection with *F. oxysporum f. cubense*, as in pot experiments. The results obtained from discoloured vascular strands of the main root were striking. In early stages of vascular discoloration, clearly associated with infection through rootlets, *F. oxysporum* was common and 7 out of 8 isolates tested were pathogenic: no other *Fusarium* spp. were isolated. More advanced stages of vascular discoloration, whether associated with die-back of the root or of unknown origin, had rather less *F. oxysporum* and only 1 out of 5 isolates tested was pathogenic. Other *Fusarium* spp. such as *F. solani* (Mart.) Appel and Wollenw. emend. Snyder and Hansen and *F. dimerum* Penzig were occasionally isolated from this type of infection. Vascular discoloration was here sometimes associated with the presence of fungi other than *Fusarium* or with that of bacteria only. With late stages of vascular discoloration, such as those found in root bases sealed off at the point of entry into the rhizome, *F. oxysporum* was again rather less abundant whereas other *Fusarium* spp. were far more numerous: only 1 out of 12 *F. oxysporum* isolates tested was pathogenic.

This evidence strongly suggests that in the field, as in pot experiments, initial root infection occurs predominantly through rootlets. It is believed

that the sequence of events is as follows. Organisms in the small vascular strands of dead rootlets are normally prevented from entering the main root, being sealed off in the manner described by Wardlaw. Where the dying rootlet has been colonized by *F. oxysporum f. cubense*, however, the host defence mechanisms are in some way inhibited and the fungus is able to penetrate the vascular strand of the main root. Here it may be checked after growing for a short distance, but usually it proceeds as far as the root base where other defensive mechanisms operate. Its chances of penetrating the rhizome are presumably governed by a number of factors, which will be considered elsewhere.

There is good evidence that other fungi entering the roots later sometimes replace *F. oxysporum f. cubense*. Thus in one relatively early stage of infection *F. oxysporum f. cubense* was abundant in the main vascular strand but had been replaced by another species of *Fusarium* in vascular strands of the rootlets through which the pathogen had clearly entered. Again, in a root through which the pathogen had recently entered a rhizome, *F. oxysporum f. cubense* was present about 10 cm. from the base, but about 1 m. farther out, at a point where initial infection had apparently occurred, a non-pathogenic strain of *F. oxysporum* was present. The smaller proportion of pathogenic strains and of *F. oxysporum* as a whole to be found in later stages of vascular discoloration is thus due at least in part to succession which leads to a corresponding increase in the number of other *Fusarium* spp. Hansford (1926*a*) noted similar successional changes in rhizomes infected with *F. oxysporum f. cubense*. The high correlation between vascular discoloration of roots and presence of the pathogen, noted in pot experiments, may well be explained by their relatively short duration which would limit the time available for successional changes. It may be pointed out that *F. oxysporum* strains classed as non-pathogenic (i.e. unable to infect rhizomes) are by no means always secondary to the pathogen; they may be associated with vascular discoloration of roots in sites where the pathogen is rare or absent.

In plantations, initial root infection was seen to occur at distances up to 1 or 2 metres from the rhizome and there seems no reason why it should not occur even farther along roots. Also, rhizome infection may occur after the pathogen has grown along a considerable length of root: it is not dependent on initiation of root infection close to the base. Roots with extensive vascular discoloration often lack surface lesions and thus rhizomes may be infected through roots whose outward appearance is normal. This situation is particularly common in light textured soils, whereas in heavy or compact soils the high incidence of cortical rot and die-back tends to obscure the mode of initial infection.

Root inoculation experiments carried out in a variety of soil types were also instructive. Small pieces of naturally infected rhizome or lengths of bamboo stem inoculated in the laboratory were placed in contact with roots of various ages; sterile inocula were used as controls. Roots were examined after 10 weeks. This method of inoculation tended to produce cortical rot even

in the controls; rot was sometimes severe and led to die-back of the root. Vascular discoloration was rare and where present had usually arisen from rootlets growing near an inoculum containing the pathogen. On the other hand, when cultures of the pathogen in soil or sawdust were used as inoculum, the incidence of vascular infection in young or moderately young roots was high and initial entry almost invariably occurred through rootlets. These experiments further indicate the importance of rootlets as points of entry for *F. oxysporum f. cubense* and also suggest that the mature cortex of main roots is generally resistant to penetration. Without experimental evidence to the contrary, however, it would be unwise to ignore the possibility that direct invasion of the cortex occurs, for instance, in badly aerated soils. The limitation of initial infection to immature tissues, such as that of rootlets, is perhaps generally characteristic of vascular wilt fungi: this is illustrated by the mode of infection of China asters by *F. oxysporum f. callistephi* (Beach) Snyder and Hansen, which has been clearly shown by Ullstrup (1937) to be confined to the root cap and region of elongation. It is noteworthy that in this disease also the root cortex is little affected even at late stages.

If, as appears likely with bananas, rootlets constitute the chief points of entry, the volume of soil from which infection can theoretically occur is very great. A banana root growing out into the soil produces short-lived rootlets, extending some 3–5 cm. in all directions, in a zone behind the tip. Thus although at any one time potential infection is limited to the region of maturing rootlets, in the course of a few months infection can theoretically occur anywhere along the whole length of root. Taking the following very approximate estimates for a mature banana plant (there is in fact great variation): average length of root, 1·5 m.; area explored by rootlets around individual roots, 0·005 sq. m.; total number of roots, 200, we obtain 1·5 cu.m. as the approximate volume of soil explored by rootlets during the lifetime of a single banana shoot. Further, since new roots are produced almost continuously throughout the life of a banana stool, the surrounding soil must be explored intensively during the course of several years. This situation helps to explain how bananas may become critically infected even with a very low soil population of the pathogen.

One further point may be considered: in view of the evidence already presented, how is radial spread of wilt in plantations to be explained? Growth of the pathogen through soil sufficiently rapid to cause the observed spread, as postulated by Hansford (1926*b*), is most unlikely: as Garrett (1944) points out, no means of active spread through soil, except in roots, has been demonstrated for vascular wilt fungi. On the other hand, the pathogen is at an early stage confined to the vascular tract, is replaced there to some extent by other fungi, and may never succeed in colonizing the outer root tissues after death owing to competition with root surface fungi. The pathogen is thus unlikely often to be in a position to pass from an infected root to a healthy one where these are in contact, and even in such circumstances infection might only occur if rootlets were present. On the whole it seems unlikely that radial

spread is to be explained solely in terms of direct root contact: more probably roots are infected from the soil as in primary outbreaks, the inoculum being derived from the breakdown of roots having vascular infection. Field evidence suggests that infected trash, where present on the soil surface, constitutes an even more potent source of infection.

ii. *Further progress of infection in the plant*

Penetration of the rhizome, build-up in its stelar tissue, and subsequent growth into the pseudostem have been fully described by earlier workers. As Wardlaw (1931) points out, the pathogen is almost certain to spread throughout the plant once the vascular tissue of the stele has been penetrated; it is, however, confined to the vessels by various host mechanisms. Ashby (1925) noted that vascular bundles in the leaf-sheath are yellow well in advance of fungal growth and that the continuity of yellow colouring often ends in streaks and dots; bacteria are not found in this region. In the present investigation bacteria were occasionally isolated in advance of fungal growth and were regularly found in later stages where vessels were darkly discoloured, confirming the observations of Hansford (1926*a*). The presence of bacteria in such early stages of infection is not surprising, for once they have entered the rhizome there is presumably little check to their movement in vessels. Drost (1912) noted that discoloration of vascular strands is often discontinuous; he detected germinating spores in minute isolated flecks and deduced that spores may be carried upwards by the transpiration stream to new points where growth occurs independently of the main advance up the vessels. The existence of discontinuous hyphal growth in vessels of the pseudostem was confirmed by plating and by direct incubation in a moist chamber at 5° C., when small isolated tufts of hyphae could be seen arising from vascular strands near the limit of hyphal advance. Discontinuous hyphal growth was never observed in the true or fruiting stem where vascular infection appeared to progress relatively slowly: even in very advanced cases of wilt the pathogen did not reach the fruits, by contrast with bacterial wilt (Moko disease) caused by *Pseudomonas solanacearum* E. F. Smith. It is tempting to believe that the rapid progress of infection in the pseudostem, as compared with that in the true stem, is associated with a faster transpiration stream in the former.

In view of the differing ability of *F. oxysporum* isolates to cause rhizome infection in pot experiments, it was thought desirable to test the ability of some to grow in vascular tissue of petioles. Five isolates, three pathogenic and two non-pathogenic, were grown in bottles of 2 per cent. malt solution for a month and the unfiltered solution of each isolate, containing numerous free spores, was inoculated into petioles of recently expanded leaves on plants 8 months old. In this operation the petiole surface was lightly swabbed with cotton wool moistened with 95 per cent. alcohol and punctured with a small rod, after which a few drops of solution were introduced with a hypodermic syringe; the wound was then sealed with adhesive plaster. For each isolate three petioles were inoculated, and these were examined after 8 weeks. There

was relatively little disorganization of tissue at the point of inoculation and a very variable development of vascular discoloration, in the main distally. Discoloration varied in colour from pale yellow to dark purple and in length from 5 to 75 cm., averaging about 30 cm.; all five isolates were recovered from discoloured vessels. There was no indication whatever that pathogenic and non-pathogenic isolates differed in ability to grow in vascular tissue under these conditions. This result, besides confirming earlier reports of the impossibility of achieving more than a very limited infection of vascular tissue by direct inoculation, further emphasizes the similarity between pathogenic and non-pathogenic forms of *F. oxysporum*. From the information at present available, the crucial difference would appear to lie in the production by the former of substances inhibiting the normal defensive mechanisms of banana tissues.

Brandes (1919) points out that the amount of fungus growth in vessels is not in general sufficient seriously to obstruct the passage of water and it is now generally believed that in many instances wilting is associated with the passage of fungal toxins in the transpiration stream, as shown for *F. oxysporum f. lycopersici* (Gottlieb, 1944). With bananas, the convenient term 'wilt' is somewhat of a misnomer since loss of turgidity is manifest only by the buckling of the petiole, often near its junction with the pseudostem. At this stage the lamina is usually turgid, although it often shows some yellowing. In plantations of 'Gros Michel' it is most usual for wilting to occur when the pathogen has grown well up into the pseudostem, but many variations occur. Interesting conditions were sometimes seen: one plant with no crown symptoms was infected two-thirds of the way up its pseudostem whereas its daughter shoot had early crown symptoms although infection was confined to the rhizome. Wilt was also seen in a young plant of the new tetraploid variety 1877 where infection was confined to the base of the rhizome. The former observation suggests that the physiological condition of the plant may affect the rate at which toxic effects develop. Field observations in fact clearly show that onset of wilt is partly determined by rate of growth: when growing fast, susceptible plants often wilt within 2–3 months of initial root infection, but when growth is checked, for instance by drought or by low temperature, wilt is greatly delayed. It is also conceivable that growth products of various strains of the pathogen vary in toxicity, but this has not been established.

Brandes describes in detail the formation of sporodochia by *F. oxysporum f. cubense* and his account has often been quoted since. He states that the pathogen growing in the petiole of a wilted leaf escapes from the vessels and soon spreads to many other tissues: sporodochia are subsequently found at the surface of the petiole and lamina, emerging through stomata. Brandes believed that the pathogen produces spores profusely in such situations and that these, becoming detached by wind, play a part in dispersal, a view often repeated in the literature. In the present investigation this aspect of the problem was approached from a different angle, an attempt being made to ascertain the status of *Fusaria* present on the lamina and petiole of banana

leaves in various conditions. Fungi were conveniently isolated by streaking suspensions of leaf surface micro-organisms in sterile water on to plates of acidified potato dextrose agar. The first isolations were made from material which had been incubated for 2 days in a moist chamber to encourage sporing: the genera found, in order of frequency, were *Fusarium*, *Cladosporium*, and *Alternaria*. *F. oxysporum* was obtained from 5 out of 15 leaves and, of 4 isolates tested, 1, from a recently wilted leaf, proved to be pathogenic. The next isolations were made directly from fresh material, when species of *Fusarium* were found to be dominant. *F. oxysporum* was the commonest species, being found on 12 out of 21 leaves in all conditions, ranging from healthy through recently wilted to dead. *F. moniliforme* Sheldon was also common. Of three *F. oxysporum* isolates tested, none proved pathogenic. Finally, isolations were made directly from buckled petioles of wilted leaves. Where the petiole surface was unbroken, *F. oxysporum* was isolated from 2 out of 6 samples, neither isolate proving pathogenic; where it was broken, *F. oxysporum* was isolated from 2 out of 3 samples, and 1 isolate was pathogenic. Of the non-pathogenic *F. oxysporum* strains isolated, several were distinct from root- or soil-inhabiting strains in having larger spores or in having a buff- or pink-coloured colony on potato dextrose agar, instead of a white or purplish one. Others were indistinguishable in appearance from such strains or from *F. oxysporum f. cubense*.

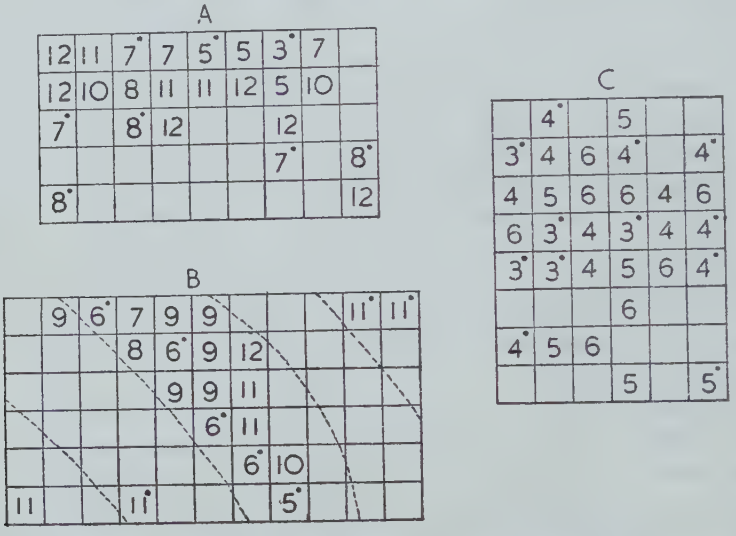
These observations, whilst not providing sufficient grounds to refute Brandes's observations, suggest that *F. oxysporum f. cubense* may not spore as freely on petiole and lamina surfaces as he supposed. It is difficult in any case to believe that, in the relatively short interval between buckling of the leaf and drying out, the pathogen is able to spread significantly from the petiole into the lamina. And towards the petiole or lamina surface it must compete with the surface population of *Fusaria* which in dying leaves may stand at least an equal chance of colonizing epidermal and sub-epidermal cells. The possible relation of spore production to dispersal will be discussed later.

iii. *Development in the plantation*

Development of banana wilt in the plantation is of practical as well as of theoretical interest since tremendous efforts were made earlier, especially in Jamaica, to check spread of the disease by various sanitation measures. It was early suspected there that the disease spread from one plant to another by root contact rather than directly through the soil, a process described by Cousins and Sutherland (1930) as 'chain-action'. However, it is clear that in diseased plantations new sporadic infections also occur and, as Wardlaw (1941) states, the controversial point with fresh outbreaks is the relative importance of such new infections and of cases due to contact with neighbouring diseased plants.

Two-monthly records of wilt were taken over periods of 1 to 2 years in several experimental banana plantations. The rate at which wilt developed varied considerably: after 16 months the incidence of diseased plants at some

sites was less than 15 per cent. whereas at two of the worst sites it had reached 56 per cent. In all plantations there was some tendency for new cases to arise next to pre-existing ones, but, especially where the development of wilt was rapid, this effect was obscured by the incidence of new sporadic infections. In view of this, it was thought desirable to establish the existence of association between affected plants by a statistical test. For two plantations in which about a quarter of the plants were affected an attempt was made to analyse the distribution of disease cases in successive rows by counting the number of

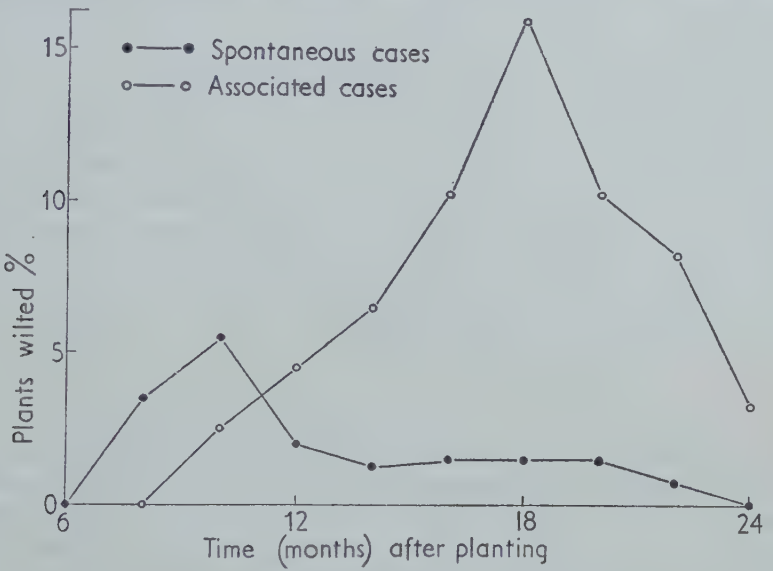


TEXT-FIG. 5. Diagrams showing the distribution of wilted bananas in portions of three plantations A–C. Each square represents one plant. In B, dotted lines represent contour drainage ditches. Numbers indicate the interval between planting and appearance of wilt, as follows: 1. Wilt arising within first period of 2 months after planting; 2. Wilt arising within second period of 2 months after planting, &c. Wilt cases judged to be spontaneous are marked thus: 3°.

'runs' of healthy and diseased plants, one diseased plant, for instance, counting as a run of 1, two successive diseased plants as a run of 2, and so on. Using a χ^2 test, it was found that the observed distributions could arise by chance only in 1:100 and 1:1,000 times respectively: there was thus excellent evidence for association between diseased plants in the rows.

From the two-monthly records of wilt cases already mentioned maps were constructed to show the development of disease. Each diseased plant was represented by a number denoting the period during which it had developed: if it had arisen in the first two-monthly period after planting it was marked 1, if in the second two-monthly period 2, and so on. A further distinction was made between plants becoming infected independently of pre-existing disease cases and those becoming infected next to such cases: the former were termed 'spontaneous' and the latter 'associated'. The term 'associated' was confined to plants which wilted more than 2 months after an adjacent plant had done

so. This interval was chosen on the basis of observation and of experimental inoculations as being the minimum time likely to elapse between initial root infection and development of wilt in rapidly growing plants. Some portions of maps so constructed are shown in Text-fig. 5. In plantation A wilt was slow to appear and associated cases likewise developed slowly; in plantation B the disease developed at a similar rate but radial spread was checked to some extent by drainage ditches along the contours. In plantation C the disease appeared early and spread rapidly: here the difficulty of discriminating between spontaneous and associated cases is obvious. Some 16 months after



TEXT-FIG. 6. Graphs showing development of wilt in a banana plantation.

planting, where the total incidence of disease was less than 20 per cent., the proportion of spontaneous and associated cases so determined was roughly equal, whereas with an incidence of 40–50 per cent. the proportion was about 1:2. This difference is no doubt mostly due to the difficulty of distinguishing between the two types of case where wilted plants are relatively crowded.

Despite the limitations of such an analysis, some interesting trends in the development of disease can be followed. In the graphs shown in Text-fig. 6, spontaneous and associated cases arising during successive 2-month periods in one of the plantations are expressed as percentages of the total number of plants and are plotted against time. Spontaneous cases first appeared 6 months after planting and the rate of appearance reached a maximum 2–4 months later, after which it declined. Associated cases reached a considerably higher peak 6 months after their first appearance and thereafter their rate of appearance also declined. The same general trend occurred in five experimental plantations, all of which had been established on sites where wilt had destroyed plantations many years earlier. It was independent of the total incidence of

disease, which was only 14 per cent. in one instance, and thus decline in the rate of spread cannot entirely be attributed to a relative scarcity of plants available for infection. It seems likely that susceptible plants are infected fairly rapidly from most of the existing centres of infection in the soil after which new spontaneous cases arise more slowly, perhaps at a rate determined chiefly by local dispersal of the pathogen. Associated cases arise at an early stage, though probably not by direct root contact, as explained earlier; an average of one or two develop around each spontaneous one. The decline in rate of appearance of associated cases is probably connected with a slow rise in host resistance; where plants are weakened by wind damage, for instance, the rate increases sharply. This effect is generally recognized by planters who find that incidence of wilt tends to be heaviest in the first year to 18 months after planting and then diminishes.

The trends just described are only characteristic of plantations where onset of wilt is relatively rapid: where it is slow, as on particular soil types, the pattern tends to be different. Here both spontaneous and associated cases arise steadily: their rate of appearance fluctuates significantly only at relatively long intervals, for instance in relation to the prevailing weather, and soon returns to normal. The ratio between the two types of case is similar to that found with more rapid outbreaks, however.

Dispersal

In an island such as Jamaica where *F. oxysporum f. cubense* has been widespread for many years it is clearly difficult to obtain much new information about dispersal. The position is complicated by the circumstantial nature of much of the evidence and by the fact that dispersal of the pathogen to new plantations is not always directly followed by appearance of wilt, owing to host resistance. Certain observations made in the course of the present work do, however, throw further light on earlier accounts of the various modes of dispersal and enable some assessment of their relative importance to be made.

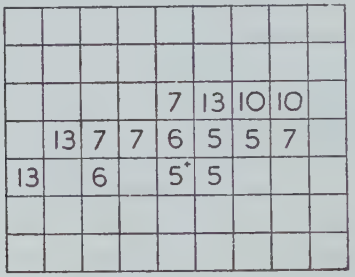
The infected banana plant is a notable direct source of infection: the pathogen was widely spread in the past by diseased planting material, for instance. Recent experiments show that partially infected suckers, when planted in soil where the pathogen is scarce, frequently produce plants which remain healthy for some time and may even produce good fruit. This condition contrasts with the early appearance of symptoms found where heavily infected suckers are used and may have given initial encouragement to planters careless in the selection of planting material. The withered pseudostem and leaves, or 'trash', of infected plants is another potent source of inoculum. Hansford (1926*b*) points out that trash used for all kinds of packing helped to spread the disease widely. Infected debris also plays a significant part in more local dispersal. In one plantation where such debris had been heaped at intervals in the banana rows, roots beneath the heaps had a high incidence of vascular discoloration and many typical early stages of infection were

found. It was discovered that roots were infected more rapidly from soil under such infected trash than from soil around an infected stool whose shoots had been removed, possibly because the pathogen returns to the soil more quickly from decaying trash than from a much bulkier decaying rhizome. The importance of trash as a source of infection is further seen after severe wind-throw, when numerous plants including some infected ones have to be cut up: the incidence of wilt in new shoots 6–8 months later is usually much greater than before.

Infected plants may to some extent be an indirect source of infection through contamination of tools such as machetes. This was shown by cutting an infected pseudostem with a clean machete: the machete surface was then found to be carrying about 3,000 viable microconidia, originating from infected vessels. A single drop of juice from the cut stem contained a similar number of microconidia. There is clearly some basis for the suggestion that where a machete used for cutting up infected plants is subsequently used for pruning healthy stools the disease becomes more widespread. A few observations were made on survival of conidia in a dry atmosphere. Suspensions of conidia were dried on glass coverslips and stored at 27° C. and at a relative humidity of 50 per cent.; viability was tested at intervals by incubating the conidia in distilled water or, for convenience in observing germination, in a thin film of 1 per cent. plain water agar. Typical results were as follows: with a dried suspension, mainly of macroconidia, obtained from a culture of *F. oxysporum f. cubense* on sterilized banana petiole, 30 per cent. were viable after 1 week, 5 per cent. after 2 weeks, and 0 per cent. after 4 weeks. With a suspension of microconidia obtained from a culture of the same isolate on malt agar, 20 per cent. were viable after 1 week, 3 per cent. after 2 weeks, and 1 per cent. after 4 weeks. Subsequent germination of microconidia was sparse and erratic, but a very small proportion germinated up to 14 weeks after drying. The period of viability of microconidia from naturally infected banana tissues was not tested, but it seems probable that tools bearing juice from such tissues could remain potentially infective for several days, and perhaps much longer.

There is good evidence that infected soil plays a part in dispersal. According to Hansford, long-range dispersal occurred in the past by the transfer to new areas of root crops taken from diseased banana plantations. This may equally well have occurred as a result of taking for planting material apparently healthy banana suckers from partially diseased plantations. It was shown by means of a pot experiment that when suckers taken from a diseased plantation are normally trimmed and planted, the small amount of soil still attached is sufficient to cause root infection. Short-range dispersal by soil is also fairly well established, and a neat demonstration of this is quoted by Cousins and Sutherland (1930). A landslide carried infected soil to a lower property and bananas subsequently planted there developed wilt and died. The importance of walking in plantations as a mode of dispersal is difficult to establish. Drost (1912), who set up certain controlled experiments in Surinam

when it was still possible to find a site free from the disease, noted infection at the ends of control plots over which labourers walked. In the present investigation it was often found that plots adjacent to heavily infected ones remained virtually free from disease for periods up to 2 years despite frequent movements of labourers throughout the area. It seems probable that the pathogen is in fact slowly dispersed by this means but that this is unsuspected because onset of the disease is delayed. It has been suggested recently that such dispersal is accentuated by a method of regular spraying for Leaf Spot (*Mycosphaerella musicola*) where long lengths of hose are dragged through the plantations. Finally, some dispersal of infected soil occurs through cultivation. This was well illustrated in a plantation where a partially diseased crop of 'Highgate' bananas was dug out, the soil harrowed, and 'Gros Michel' planted. At a position where one former diseased stool had been overlooked, such



Text-fig. 7. Diagram showing the distribution of wilted bananas in a portion of a plantation around the site of a previously diseased stool (marked +), 2 years after replanting. Notation as in Text-fig. 5.

places not usually being replanted, 4 plants wilted almost simultaneously after 9 months and 11 further cases developed within 2 years (Text-fig. 7). Distinction should be made between uniform cultivation of a site after removal of a partially diseased crop and inter-row cultivation of a standing crop. The latter process, although it may gradually disperse infected soil over a period of time, is not followed by a sudden increase of disease as with the former; it is probable that heavily infected soil is confined to the immediate vicinity of disease cases and is little disturbed by inter-row cultivation. Other factors besides dispersal of the pathogen are, however, involved in cultivation and these will be discussed in a subsequent paper.

The opinion of Brandes (1919) that wind-borne spores play a significant part in dispersal has already been mentioned. In Porto Rico Brandes exposed agar plates for half an hour between rows of diseased banana plants and obtained between 1 and 30 colonies of *Fusarium oxysporum* per plate. He believed that these were colonies of the pathogen, but in view of the evidence given earlier it seems at least as probable that they were colonies of saprophytic strains. Brandes also believed that the effectiveness of conidia as agents of dispersal was limited by their short survival, and whilst this is a reasonable suggestion he clearly underestimated the capacity of conidia to survive, at

least under dry conditions. However, in any discussion about dispersal properties of spores other than duration of viability must be considered; in particular, the slimy nature of *Fusarium* spores would appear to favour dispersal by water rather than by wind. That *Fusarium* spp. are not in fact significantly dispersed by wind, at least in Britain, is suggested by the results of spore-trapping in arable fields: in the course of examination of hundreds of slides exposed during successive summers, only 1 or 2 typical macroconidia of *Fusarium* were observed (Gregory, 1954). Again, attempts in Jamaica to repeat Brandes's observations were unsuccessful: *F. oxysporum* failed to develop on agar plates exposed under conditions similar to his, although other *Fusarium* spp. very occasionally appeared. With banana wilt, there is thus not only serious doubt as to whether conidia produced by the pathogen are adapted to wind-dispersal but doubt as to the extent to which such conidia are formed on the surface of affected plants (p. 313). Such considerations, taken in conjunction with the great dilution of inoculum which would occur if conidia were in fact transported by wind, suggest that such a mode of dispersal is relatively unimportant.

The importance of long-range dispersal of the pathogen in soil and plant debris by flood water had been stressed by Smith (1932). Plantations on alluvial soils in Jamaica were in general free from the disease until severe flooding occurred: good examples are the Rio Grande valley in Portland and the plains adjoining the Rio Cobre in St. Catherine. It is noteworthy that banana cultivation in hill country was a major factor leading to soil erosion, consequent severe flooding, and thus to spread of banana wilt. On a smaller scale, local flooding of streams is still responsible for new outbreaks of the disease. There is also good evidence that flooding in plantations after heavy rain is an important factor in local dispersal. Opinions vary about the importance of irrigation water in spreading the pathogen. Smith notes that many cases of banana wilt appeared near irrigation canals in St. Catherine, and also that there was a considerable check in the incidence of disease in 1931 when heavy rainfall made irrigation unnecessary for long periods. Many planters, on the other hand, claim that irrigation water is not particularly suspect. Methods of irrigation vary considerably, and the extent to which dispersal occurs within a plantation may well be related to the volume and rate of flow of the water.

The Saprophytic Phase

Although it is possible that some spores of *F. oxysporum f. cubense* borne on petioles of infected plants are washed into the soil, the pathogen does not markedly increase there until the plant has fallen and begun to decompose (p. 321). During this process, which is complete within a few months, the pathogen is probably replaced to some extent by other fungi. When small pieces of naturally infected rhizome were buried in plantation soil the pathogen was readily isolated after 4–5 weeks; after 8–9 weeks, however, replacement by fungi such as *Fusarium solani* and *Trichoderma viride* Pers. ex Fr. was

extensive or even complete. The period of survival of the pathogen in infected roots or rhizomes is further limited by their rapid decay. By contrast, the pathogen survives in soil for long periods. A good instance of this is provided by the experience of a Jamaican planter who, at a site abandoned through banana wilt 20 years before, planted 100 'Gros Michel': only 2 survived to the stage of flowering. This ability for long survival was recognized by Hansford (1926*b*), who stated that all land once infected with Panama disease can be considered as permanently useless for the cultivation of 'Gros Michel'. Amongst closely related pathogens, similar lengthy survival in soil has been reported for *F. oxysporum f. lini* and *F. oxysporum f. conglutinans* (Garrett, 1944).

The factors affecting incidence of banana wilt in the field are complex and hence any estimate of the soil population of the pathogen involving counts of diseased plants is virtually worthless. Further, for reasons given earlier, this population cannot be determined by soil plating methods. However, by using the more standardized conditions for disease development of a glasshouse test, the natural infective capacity of a soil can be estimated by growing banana plants in representative samples for 3 months and then counting the number of rhizome infections. Soil dug from a depth of 0–6 in. was transported in clean sacks from various sites to the glasshouse, where it was well mixed, sieved to remove root fragments, and used to fill 12 pots, each with 10 kilos. Small 'Gros Michel' suckers were then planted and conditions maintained as described earlier. The number of rhizome infections varied greatly with the origin of the soil, averages per plant ranging from 0 to 6. McKee and Boyd (1952), who used a similar type of method to estimate the infective capacity of soils containing *Fusarium coeruleum* (Lib.) Sacc., point out that possible variations in virulence of the pathogen and in the biological characteristics of different soils make it difficult to relate soil infectivity to soil population. With bananas, the latter factor may be discounted to some extent, for when various different soils lacking the pathogen were similarly inoculated the number of rhizome infections obtained was also similar. Further, striking differences were often found between closely adjacent sites having the same soil type. As an approximate indication of the abundance of *F. oxysporum f. cubense* in natural soils, populations have been estimated in viable spores per gramme, the basis for these estimates (necessarily very tentative) being the number of rhizome infections given by various known inoculum dosages of the pathogen in a light, alkaline loam. The lowest population level at which the pathogen was detected in the soil by means of this test corresponded to a dosage of two viable spores per gramme of soil (expressed thus: 2/g.).

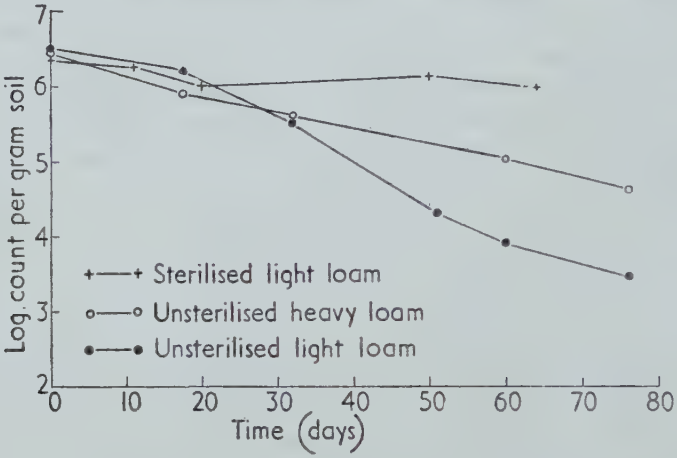
By this method certain general trends in soil population of the pathogen were followed. *F. oxysporum f. cubense* cannot be detected (< 2/g.) in soils which have never borne bananas and is not usually detected at sites abandoned through the disease more than 8 years before and not subsequently replanted with bananas. A small population must in fact be present at these sites, for when 'Gros Michel' is replanted wilt soon develops. In such instances no

increase of the pathogen is found before plants wilt or directly after they have done so, but an increase (up to 50/g.) can be detected immediately around plants a few months later. As more shoots wilt and collapse, the soil population increases further until after 1 or 2 years it may be relatively large around diseased plants (up to 300/g.). Dispersal to soil between infected plants is somewhat delayed, but here, too, numbers increase considerably. When diseased plants are removed the pathogen rapidly diminishes in numbers, counts of about 10/g. being found after 2–3 years. If a site bearing diseased 'Gros Michel' is replanted with 'Lacatan' the population of *F. oxysporum f. cubense* declines, but not to the extent that it does if another crop is planted or the site is left fallow. Under some conditions, apparently, the population around 'Lacatan' remains relatively high (about 100/g.): this was the case with a plantation where the variety had been growing continuously for 9 years. *F. oxysporum f. cubense* was detected (about 5/g.) around a 3-year-old resistant seedling variety at a site where only slightly diseased 'Gros Michel' were present 8 years before. The pathogen may thus be maintained to some extent in plantations of resistant banana varieties. By contrast, the pathogen could not be detected in certain 'Gros Michel' plantations still virtually free from wilt after several decades of continuous cultivation.

The rapid decline in soil population of *F. oxysporum f. cubense* after removal of the affected crop is striking: on the basis of the approximate estimates quoted, the population apparently falls to about 3 per cent. of its peak value within 3 years and to below 0·5 per cent. within 8 years. The method used gave no indication of the extent to which numbers declined thereafter. The other observations are probably to be interpreted in terms of host-parasite relationships, which will be considered more fully in a subsequent paper. Briefly, there is evidence that soil conditions influence root infection and that banana varieties resistant to wilt suffer a certain amount of root infection with *F. oxysporum f. cubense*. It is suggested that, in general, sites abandoned through wilt have soil conditions favouring root infection and that when resistant varieties are subsequently planted their roots become infected to some extent. The soil population of the pathogen remains at a relatively low level because infection is usually confined to the roots, whereas with susceptible varieties it often spreads to the rhizome and pseudostem. In such instances the pathogen does not depend on a saprophytic existence for survival. In old healthy 'Gros Michel' plantations, however, it is suggested that soil conditions are relatively unfavourable to root infection and that, as a result, the pathogen does not increase to an extent detectable by the method used. That *F. oxysporum f. cubense* is not entirely absent is shown by the rare, scattered incidence of wilt in these plantations; and in view of its scarcity under these conditions it seems unlikely that the pathogen is maintained to any significant extent by saprophytic colonization of dead banana roots. The apparent inability of the pathogen to colonize dead roots in pot experiments has been noted earlier. Stover (1953), on the basis of experiments carried out with soil in glass tumblers, believed that *F. oxysporum f. cubense* colonized dead banana roots

and survived in them for at least 9 months. Here, however, the initial population of the pathogen was very high (over 1×10^5 per g. soil): in natural soils with low populations of the pathogen saprophytic *Fusaria* would have at least an equal chance of colonizing dead roots and it could not be assumed without testing that strains of *F. oxysporum* present in them after 9 months were pathogenic.

In the course of glasshouse experiments it was found necessary to study the fate of *F. oxysporum f. cubense* in pots of soil into which the fungus had been introduced either as a spore suspension or as a small amount of sawdust inoculum. Conditions were very different from those obtaining in natural



TEXT-FIG. 8. Graphs showing survival of *F. oxysporum f. cubense* spores in pots of soil.

soils since there were wide fluctuations of moisture content, influenced by daily watering, and of temperature. The inoculum, well dispersed in the soil, was sufficiently heavy to give counts measurable by plating for many weeks. Some typical results obtained from soils inoculated with a spore suspension are illustrated in Text-fig. 8 where the logarithmic count per g. soil is plotted against time. In the unsterilized light loam there was a rapid decline in numbers throughout the experiment, whereas in the same soil sterilized by autoclaving numbers remained relatively high. The fate of the pathogen in heavy loam was only tested in one experiment when the rate of decline was less than that in the light loam. Stover (1953), who studied survival of *F. oxysporum f. cubense* in tumblers of soil, often obtained an initial increase in numbers, probably because the inoculum contained unassimilated nutrients. Thereafter he obtained marked reductions in count which he attributed to exhaustion of food. If this explanation is correct, the immediate decline in numbers occurring after inoculation with spores alone is readily understood; and further, lack of competition for nutrients could account for the observed maintenance of a high population in sterilized soil. The rates of decline which

Stover observed in pots of soil partially buried outdoors are comparable to those recorded in Text-fig. 8; he too found that decline was more rapid in the lighter of the two loams tested. These observations are of interest in showing that *F. oxysporum f. cubense*, when introduced into unamended soil and held under artificial conditions, rapidly decreases in numbers; a similar but less rapid decline occurs with natural soils after removal of wilted plants. It is unfortunate that plating methods can yield useful information only where initial counts are relatively high; even so it is only possible to follow the earlier stages of survival.

As an adjunct to this study, it was thought desirable to determine whether the natural soil population of *F. oxysporum f. cubense* was related in any way to that of other soil fungi and in particular to that of non-pathogenic strains of *F. oxysporum* with which, it seemed probable, the pathogen had often been confused. Soil taken from various depths was plated directly (Warcup, 1950) with Dox's agar, using an average of 0·004 g. soil per plate. After incubation at room temperature (28–30° C.) for 10 days, the relative abundance of various genera such as *Fusarium*, *Trichoderma*, and *Aspergillus* was recorded and at the same time the number of *F. oxysporum* colonies was counted. An approximate estimate of the soil population of this fungus at each site was made on the basis of counts from 5 to 15 plates. Surface soil provided the most satisfactory comparisons and for convenience results obtained from soil samples taken at 1 in. and 3 in. are grouped together. The majority of samples were taken from alkaline soils, and in these the population of *F. oxysporum* was found to vary considerably with texture. Heavy loam or clay soils had counts of 0–100 (average 30) per g., whereas light to medium loams generally had counts in the range 100–800 (average 400) per g. Two heavy acid soils were found to have 1,200 per g. These results, as far as they go, agree well with those of Reinking and Manns (1933) for the distribution of *F. oxysporum f. 3* in Central American soils, which fungus was less abundant in clay than in lighter textured soils, and less abundant in alkaline than in acid soils. The general similarity of counts obtained from comparable soil types in the two investigations in fact suggests that there is a reasonable correspondence between the fungi classed respectively as *F. oxysporum* and *F. oxysporum f. 3*. It may further be noted that Reinking and Manns most commonly used a dilution of 1/250 for plating and acidified the medium to pH 4·0–4·5, so that their dilution method was if anything rather less sensitive in detecting small populations than the direct method used here. The failure, mentioned earlier, to obtain pathogenic isolates from plates prepared by the latter method cannot therefore be ascribed to its lower sensitivity.

The soil population of *F. oxysporum*, though related to soil texture and reaction in the ways indicated, was independent of the state of health of the plantation. Thus the fungus was abundant in some plantations severely affected by wilt within 1 or 2 years of planting and was at least as abundant in some others where 'Gros Michel' had remained virtually free from wilt for 20 years or more. Equally, in plantations where the fungus was not detectable

in the soil, the incidence of wilt varied from extremely light to severe. Other *Fusarium* spp., of which *F. solani* was the commonest, and *Trichoderma viride* were found in nearly all plantation soils; their abundance was not correlated with the incidence of wilt. There was, however, some tendency for *Aspergillus* spp. to be more frequent in soils of severely affected plantations. If *Aspergillus* spp. are relatively tolerant of fluctuating soil moisture content, as suggested by Stover (1953), their frequency in severely affected plantations may be significant, for variable soil moisture is almost certainly an important factor predisposing bananas to wilt.

Where simultaneous estimates of population were made for non-pathogenic strains of *F. oxysporum*, by plating, and for *F. oxysporum f. cubense*, by the pot test, widely differing results were obtained. In healthy plantations or in those very recently affected by wilt the former often preponderated by a ratio of several hundred to one, whereas in plantations affected for over a year the two were more equal in numbers, ratios of 5:1 or even 1:1 being obtained from soil around diseased plants. If such ratios are only approximately correct, the difficulty of obtaining pathogenic isolates by plating soil is readily understood. In all except heavily infected soil around diseased plants the chance of obtaining the pathogen is negligible; around such plants the chance in particularly favourable circumstances may be about even.

In the light of these results it is interesting to consider those of Reinking (1935), who studied the population of *F. oxysporum f. 3* in various Central American soils by plating surface soil taken from the vicinity of diseased stools. He suggests again that size of population is related to soil texture and also, in general, to severity of wilt. Since, however, the ratio of pathogenic to non-pathogenic strains of *F. oxysporum* around wilted plants apparently varies, soil plating cannot have given an accurate guide to the numbers of the pathogen alone. If nevertheless the count so obtained had given a rough indication of this population, it seems reasonably certain that this would have represented the build-up of the pathogen around wilted plants and not the population at the time of the initial outbreak, which is probably very much smaller than Reinking believed. These observations are not meant to detract from the interest and importance of Reinking's work and can at the most lead to changes of emphasis. It was noted in the current investigation, for instance, that build-up of the pathogen around wilted plants, as measured by the pot test, tended to be less in heavy soils than in light ones, which accords well with Reinking's views that the pathogen dies out at varying rates in different soil types. However, this differential effect of soil type is probably most marked in relatively early stages of saprophytic survival: it appears that in all soil types the pathogen declines markedly in the first year or two and then persists in very small numbers almost indefinitely. There is no doubt that incidence of banana wilt is correlated, amongst other things, with soil texture, and since the soil population of *F. oxysporum*, as estimated by soil plating, is similarly correlated with soil texture, it is perhaps not surprising that Reinking found a relation between all three. The fact that the soil population

of *F. oxysporum* was not found to be correlated with incidence of wilt in the present investigation might be explained partly by the fact that only a small proportion of soil samples were taken from around affected plants, whereas Reinking's sampling was confined to this position. It is possible, for instance, that *F. oxysporum* temporarily increases in numbers around diseased stools, where unusually large amounts of organic matter are breaking down. Apart from these considerations, it seems very probable that the factors determining incidence of wilt do not entirely correspond with those determining abundance of *F. oxysporum*, although they may have something in common.

A tentative conclusion is that non-pathogenic strains of *F. oxysporum* are soil inhabitants, as defined by Garrett (1950), occurring in a wide range of soil types. Their abundance is determined to some extent by soil texture, but they are not greatly affected by the presence of bananas, whether healthy or suffering from wilt, except in so far as they colonize dead or moribund tissues. *F. oxysporum f. cubense* also occurs in a wide range of soil types, but its abundance is greatly affected by the presence of bananas. Despite its relative scarcity in soil it has a marked competitive advantage over saprophytic strains of *F. oxysporum* since it can invade susceptible plants and grow extensively in them. After collapse of the plant and subsequent breakdown of its tissues, the pathogen returns to the soil in greatly increased numbers. Here, in the absence of its host, *F. oxysporum f. cubense* rapidly declines in numbers and after about 10 years cannot be detected by any test so far devised, although its continued survival is well established by many field observations on the incidence of banana wilt. The fact that the pathogen shows this rapid decline in soil population and moreover is unable to colonize dead banana roots to any great extent, as demonstrated earlier, suggests that it has a relatively poor competitive saprophytic ability. The pathogen thus has attributes both of root- and soil-inhabiting fungi, though by reason of its long survival in soil it seems preferable to classify it with the latter.

The mode of survival of *F. oxysporum f. cubense* in the absence of bananas is unknown. It seems unlikely that the fungus is maintained to any great extent on roots of plants unrelated to the banana, and the opportunity to persist on roots of related plants such as *Heliconia* only occurs where secondary forest containing such plants is allowed to develop, as in Surinam (Drost, 1912). The sclerotia formed by some isolates in culture do not appear to be sufficiently resistant to survive for long periods in soil and their presence in natural soils has not been demonstrated, so far as is known. The survival of conidia in soil is almost certainly short, as with *F. oxysporum f. vasinfectum* (Subramanian, 1950). Thus by elimination it seems probable that the pathogen exists saprophytically as vegetative mycelium or, in unfavourable conditions, as chlamydospores. Buxton (1954), working with *F. oxysporum f. gladioli* (Massey) Snyder and Hansen, has shown that heterocaryosis accounts for much morphological variability. When conidia were germinated on plain water agar, hyphal fusion, leading to association of different nuclei, occurred frequently. It seems possible that by a similar process nuclei with different

potentialities for determining pathogenicity on the one hand and saprophytic ability on the other become associated in hyphae under natural conditions, but this has not yet been established. Buxton points out that if such heterocaryons are present in soil, roots of a host plant could provide a selective environment for the nuclear types present and suitably pathogenic strains would be favoured.

In this connexion two points of interest arise from the present investigation. Firstly, although there was opportunity to make only a few adequate comparisons, it was established that *Fusarium* isolates from wilted bananas may differ somewhat in pathogenicity. In a pot test one newly obtained isolate gave a total of 55 rhizome infections in 12 'Gros Michel' plants whereas an older isolate gave 106, the difference being statistically significant. This difference in pathogenicity is enhanced by the fact that the former isolate produced far more viable spores in the same weight of inoculum. Whether or not selection of fungal strains occurs within the plant, this observation suggests that *Fusaria* entering from the soil are heterogeneous with respect to genetic material determining pathogenicity. Secondly, although soil isolates were all classed as non-pathogenic on the basis of a test involving two or three plants, yet in a few instances some rhizome infections were produced. It seemed possible that more comprehensive tests, for which unfortunately there was not time, might have established the existence of strains with very low pathogenicity. If the existence of such strains could be proved, this would help to explain the prolonged survival of the pathogen and, incidentally, would throw interesting light on its relation to other strains of *F. oxysporum*.

Summary

The pathogen, *F. oxysporum f. cubense*, is very difficult to isolate from soil by plating but can be detected in soil by a suitable host test. When small bananas are grown in pots under appropriate conditions, pathogenic isolates of *F. oxysporum* produce rhizome infections whereas other isolates do not. Of *F. oxysporum* isolates obtained by plating, the proportions from various sources proving pathogenic were as follows: from the rhizome or pseudostem of plants showing typical wilt, 91 per cent.; from variously affected roots, 29 per cent.; and from soil, 0 per cent.

It is suggested that the banana pathogen, like other parasitic forms of *F. oxysporum*, has a limited host range; however, it causes a limited root infection of *Heliconia psittacorum* and may possibly originate on plants such as this related to the banana.

Initial infection often occurs through rootlets; the pathogen apparently cannot penetrate the cortex of main roots except through the vascular strand of rootlets, at least in well-aerated soils. Infection does not occur through dead roots. The further progress of infection is described; in later stages the pathogen is replaced to some extent by other fungi.

An account is given of the development of wilt at sites replanted after earlier abandonment through the disease. New cases arise both spontaneously

and in association with pre-existing ones, the rate of appearance of each type tending to decline after an initial rise.

Various modes of dispersal of the pathogen are discussed: dispersal by flooding is still important in Jamaica.

F. oxysporum f. cubense survives for a relatively short period in infected plant material, owing to rapid decay, but it survives for many years in soil. The infective capacity of certain soils, thought roughly to indicate abundance of the pathogen, was determined by a host test. There is a striking increase in the population a few months after wilt appears and a decline after infected plants are removed. Wide variation was found in the ratio of non-pathogenic to pathogenic strains of *F. oxysporum* in soils: usually it is very high.

The possible mode of survival of the pathogen in soil is discussed.

Acknowledgements

The writer wishes to acknowledge assistance received from officers of the Department of Agriculture, Jamaica, and especially that from Mr. A. G. Naylor, who supervised many field experiments. He is further much indebted to colleagues in the West Indian Banana Research Scheme and to Dr. S. D. Garrett and Dr. N. F. Robertson for advice and discussion. Acknowledgements are also due to the following: Dr. W. L. Gordon, for identifying *Fusarium* isolates; Dr. W. C. Snyder, Mr. G. B. Wallace, Mr. N. W. Simmonds, and Mr. W. G. Kovachich, for providing various *Fusarium* cultures; the Curator, Royal Botanic Garden, Kew, for providing plants of *Heliconia psittacorum*; Professor P. H. Gregory, for supplying unpublished data about spore-trapping; Dr. J. Wishart, for advice about a statistical problem; and Mr. F. T. N. Elborn, for taking the photographs.

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EXPLANATION OF PLATES

Illustrating J. Rishbeth's paper on '*Fusarium* wilt of bananas in Jamaica. I. Some observations on the epidemiology of the disease'

PLATE VI

Roots from small 'Gros Michel' bananas, grown in pots of soil inoculated with *F. oxysporum f. cubense*, cut longitudinally to show vascular infection progressing (right) towards the rhizome. (×2.)

A. Early stage, arising from one rootlet.
B. Later stage, arising from several rootlets.
C. Advanced stage, arising from several rootlets, showing cortical rot.

PLATE VII

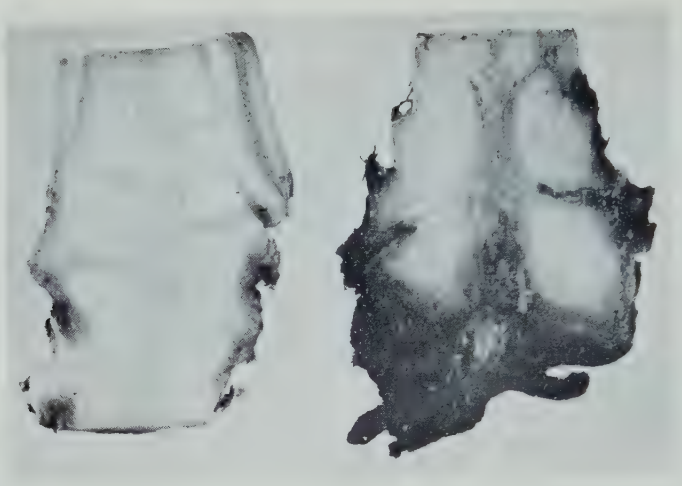
A. Rhizomes of *Heliconia psittacorum* cut longitudinally to show root bases and central vascular tissue. (×3.)
Left: plant not inoculated; tissues healthy.
Right: plant inoculated with *F. oxysporum f. cubense* and receiving ammonium sulphate; rhizome with basal rot and discoloured vascular tissue.
B. Root systems of *H. psittacorum*. (×½.)
Left: plant not inoculated; root system healthy.
Right: plant inoculated with *F. oxysporum f. cubense* and receiving ammonium sulphate; many roots dead and rootlets withered.

A

B

C

J. RISHBETH



A

B

J. RISHBETH

The Relative Growth Rates of Three Plankton Diatoms in Relation to Underwater Radiation and Temperature

BY

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(*Department of Botany, University College, Khartoum*)

With Seven figures in the Text

Abstract

Relative growth rates of three freshwater plankton diatoms—*Asterionella formosa*, *Fragilaria crotonensis*, and *Tabellaria flocculosa* var. *asterionelloides*—are described from cultures suspended at various depths and during several seasons in the lake Windermere. Seasonal variation in rates recorded near the surface (1 m. depth) is interpreted in terms of seasonal changes in temperature and day-length. Rates recorded for *Asterionella* and *Fragilaria* are generally similar, but are approximately twice the rates obtained with *Tabellaria*. Depth profiles of relative growth rates are of similar form in all species, and normally show light-saturation near the surface. The shape of profiles for *Asterionella* is in good agreement with estimates of photosynthesis integrated over the growth periods. The parallelism between photosynthetic and relative growth rates of *Asterionella* is further illustrated from laboratory experiments: an approximate interconversion, under certain conditions, is given.

Introduction

RELATIVE growth rates of phytoplankton organisms have been used by various authors (e.g. Boysen Jensen, 1914; Juday, 1940; Harvey, 1950) to assess rates of organic production in natural water-bodies, either directly or using the indirectly related concept of the 'turn-over period'. Such calculations have invariably been of an extremely approximate character. Although a number of investigations of the relative growth rates of phytoplankton organisms have been made in relation to ecological problems, it is difficult to make any direct comparison between such data and the growth of natural populations. An approach to natural conditions is afforded in studies of the growth of algae in bottles suspended in the water-column (Whipple,

[1] This paper forms part of a thesis submitted for the degree of Ph.D. in the University of Leeds.

1896; Gran, 1927, 1931, 1933; Gaarder and Gran, 1927; Lönnerblad, 1929; Loose, Pearsall, and Willis, 1934; Gauld, 1949; Lund, 1949; Grim, 1950; Riley, 1952), but such observations have not been adequately compared with corresponding photosynthetic behaviour or of growth under standardized laboratory conditions. In consequence a detailed interpretation is rarely possible.

In this paper such an interpretation is attempted. It is based upon comparative measurements of the relative growth rates of three species of fresh-water plankton diatoms, exposed as cultured populations under field conditions. These measurements are used for inter-specific comparisons and for comparison with photosynthetic behaviour under similar conditions. The latter comparison is further illustrated by data from laboratory experiments.

Methods

The species investigated were the diatoms *Asterionella formosa* Hass., *Fragilaria crotonensis* Kitton, and *Tabellaria flocculosa* var. *asterionelloides* (Grun.) Knud. The majority of experiments utilized clones of these algae isolated from Lowes Water, Esthwaite Water, and the Kendal Canal (near Windermere) respectively. For *Asterionella* and *Tabellaria* occasional comparisons were made with the relative growth rates of non-clonal populations of the same species isolated from Windermere. No significant differences were found, and data from the Windermere 'strains' are not discussed further. A few experiments were performed using direct samples of a natural *Asterionella* population present in Windermere (North Basin) in the spring of 1952. Otherwise the algae were grown as unialgal cultures in the medium No. 10 of Chu (1942) made up in Windermere lake water, and modified by the use of the iron and manganese sources recommended by Rodhe (1948) and the inclusion of 0·5–2·0 per cent. of an extract from Windermere mud. In most field exposures media containing different concentrations of mud extract (within the above range) were used, to show that this component was present in optimal or near-optimal concentration. The omission of the sodium carbonate component of the medium—giving a pH (*c.* 7·4) more similar to that of the unmodified lake water (*c.* 6·9)—was found not to influence the relative growth rates recorded.

The inoculation density (normally 10–100 cells/ml.) was such that the maximum densities resulting from growth did not exceed the densities characterizing the upper limit of the exponential growth phase as observed in laboratory experiments. The cultures were inoculated from actively growing parent cultures and allowed to stand overnight in darkness before exposure the following day. After this dark period it was found that further cell division in darkness was negligible, and so a correction for such 'dark division' was not required (cf. Gaarder and Gran, 1927; Lund, 1949).

In field exposures flat-sided, soft-glass bottles of approximately 300-ml. capacity were used, supported horizontally by their necks and bases on an aluminium framework. Eight bottles could be carried by each framework.

shading of the bottles by the latter was small or negligible. The frameworks were suspended at various depths in Windermere from the buoy in the North Basin, which was also used in photosynthesis experiments (Talling, in prep.). The normal depths used were 1 m., 2·5 m., 5 m., 7 m., and 9 m. In 1951 some bottles were also exposed at 0·5 m., and these may have occasioned some appreciable shading of the bottles at 1 m. Otherwise shading effects between the sets of bottles was probably negligible.

Relative growth rates were computed, based upon increase in cell number (cell divisions per week) as an average rate for the exposure period, which was approximately 1 week. The growth of a species at each depth was normally obtained from cultures in duplicate or triplicate; unhealthy cultures were discarded. Cell counts were made by the Utermöhl (1931)-Lund (1949) technique, one count being made per culture. The general design of the field experiments was based upon the procedure adopted by Lund (1949).

Since the cultures could not be shaken in the lake (apart from random wave action), they are open to criticism as 'stagnant' cultures. The low cell densities used would, however, reduce effects arising from cell sedimentation. The relative growth rates recorded, under favourable environmental conditions, are also high (equivalent to rates under continuous illumination of the order of 2 divisions/day). This suggests that any considerable retardation of growth by the conditions of culture is unlikely.

Laboratory experiments utilized a refrigerated chamber and general technique of photosynthesis determination (Winkler method) which will be described elsewhere. Light intensities incident upon the algal suspensions were varied by interposing filter-papers between the light source and bottles. The bottles were screened from diffused lateral light by cylindrical shields. Light intensities were measured upon a relative scale using a selenium photocell, care being taken to avoid 'curvature errors' of the instrument. The same glass bottles were used in measurements of relative growth rates and in short-period photosynthesis determinations. Material used in the two types of determinations was derived from a common parent culture. Relative growth rates in continuous illumination were calculated from the gradient of a plot of logarithms of cell density against time, derived from exponentially growing cultures. The cultures were sampled daily, or twice daily, over a growth period of 3–4 days. Each experimental condition was represented in duplicate. Owing to the method of illumination by a fluorescent tube below the cultures, convection currents were created within the cultures that maintained most cells in slow circulation. The bottles were shaken twice daily. A preliminary experiment showed that the highest light intensity used was sufficient to give complete, or nearly complete, light-saturation of photosynthesis at the highest temperature used.

Relative Growth Rates at 1 m. Depth

Relative growth rates recorded at 1 m. depth were normally the maximum rates obtained during the field exposures. Variation of these rates in relation

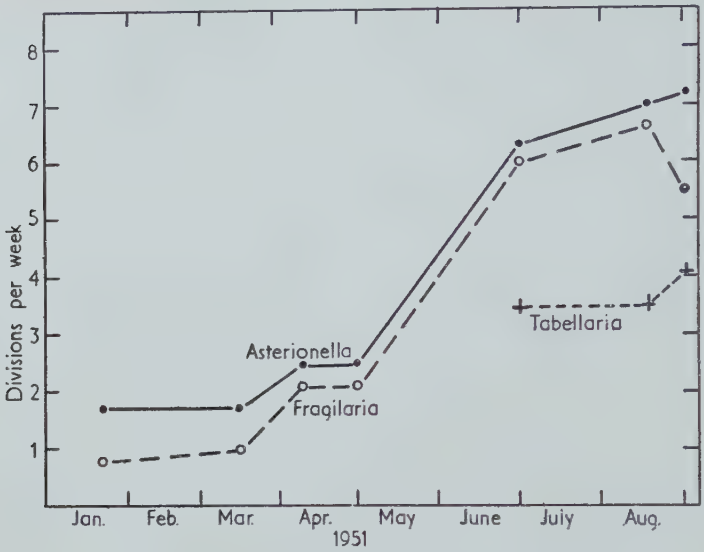

FIG. 1. Mean relative growth rates of three species in cultures suspended at 1 m. depth in Windermere North Basin during 1951.

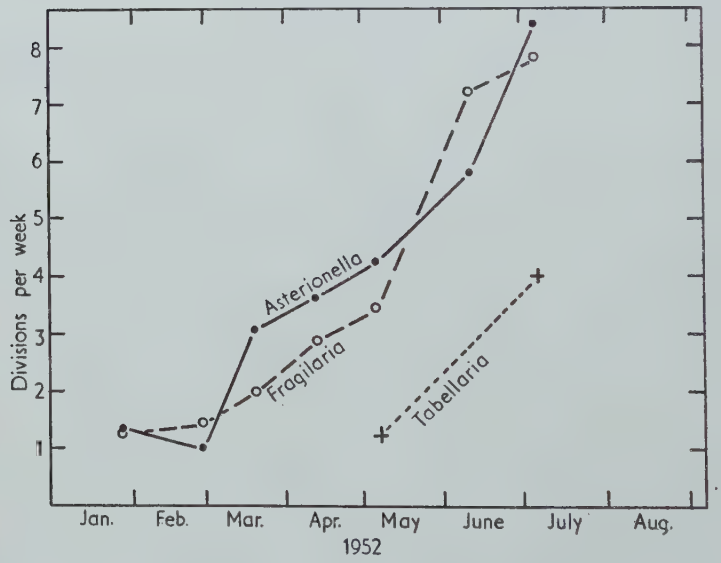

FIG. 2. Representation, as in FIG. 1, of data obtained during 1952.

to season is shown in Figs. 1 and 2. The experiments were continued over the first parts of two years, 1951 and 1952. In general the rates recorded for *Asterionella* and *Fragilaria* are of similar magnitude, whereas the rates for *Tabellaria* are approximately half those recorded for the other species. This comparison is in general true for rates measured at other depths, and also for

growth of the three species in the laboratory with the same culture medium as in field experiments.

The increase in the relative growth rates recorded for *Asterionella* between winter and summer conditions may be related to photosynthetic behaviour—which will be described in detail in a separate paper (Talling, in prep.)—as follows. Since light-saturation of photosynthesis normally occurs at light intensities that are low in comparison with the intensity of surface-incident

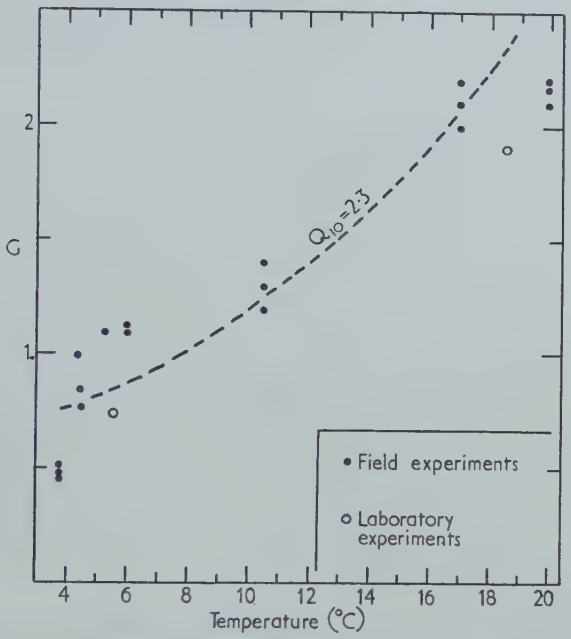

FIG. 3. Relative growth rates (*G*) of *Asterionella*, recorded at 1 m. depth in Windermere North Basin during 1952 calculated as cell divisions per 24 hours of daylength and expressed in relation to temperature. Each experimental point is based upon one culture. Circles denote relative growth rates measured under conditions of light saturation in the laboratory expressed in a parallel manner (divisions per 24 hours of illumination).

radiation (spectral region 400–700 mμ), and since approximately one-half of this latter intensity is present at 1 m., the photosynthesis of cells at 1 m. can be expected to be essentially light-saturated over almost the entire daylength. Neglecting phenomena of 'subsurface inhibition', which are rarely appreciable at 1 m. depth in Windermere, and respiration losses which are of a small order of magnitude (Talling, in prep.), the relative growth rates shown in Figs. 1 and 2 can be expected to parallel light-saturated rates of photosynthesis continued over the available daylength. Since such photosynthetic rates are primarily determined by temperature in these experiments, the seasonal variation recorded in Figs. 1 and 2 should be primarily a function of daylength and temperature. Both these factors would cause an increase in the relative growth rates recorded in summer, as compared to winter, conditions.

The above interpretation is illustrated in Fig. 3, which shows the rates

obtained with *Asterionella* during 1952 computed per unit period of daylength and plotted against temperature. Mean daylength values were derived from continuous radiation records for the experimental periods, in relation to intensities above 16 kiloerg/cm.^{2} sec. in the spectral region 400–700 mμ. This representation assumes that relative growth rates under discontinuous illumination will be reduced, in comparison with rates under continuous illumination, in proportion to the fractional illumination period. Such a relation was found in laboratory experiments with an artificial 12-hour daylength. Similar results have been recorded in other studies of algal growth, although deviations under rapidly alternating illumination have been described (Iggena, 1938; Behrend, 1948).

Fig. 3 shows that the effect of temperature upon the relative growth rate is compatible with a Q_{10} value of 2·3, which has been found applicable to the variation of light-saturated photosynthetic rates with temperature (Talling, in prep.). The absolute values of the relative growth rate per unit period of illumination are also similar to light-saturated relative growth rates obtained in laboratory experiments at the same temperatures, using continuous illumination. Two such values, obtained from an experiment described later (Fig. 6), are inserted in Fig. 3. With the lowest relative growth rates recorded in Fig. 3, increase in cell number may possibly diverge markedly from other indices of growth. The data are, however, consistent with the expectation that the mean relative growth rates of cells at 1 m. depth are primarily determined by daylength and temperature. The onset of thermal stratification in the lake can be expected to result in a sudden increase in the relative growth rates, whereas the influence of increasing daylength will extend over a longer and mainly earlier period.

The rates recorded for *Asterionella* and *Fragilaria* early in 1951 are generally lower than the corresponding values in 1952. The latter are probably the more reliable, as possible shading by cultures at 0·5 m. was never present, and the 1952 values for *Asterionella* are in good general agreement with values obtained previously by Lund (1949).

In 1952 four field exposures were made using direct samples of a natural population of *Asterionella* present in Windermere North Basin. Exposures in January and February yielded very low relative growth rates (of the order of 0·4 division/week), but in this period accurate measurements were difficult to obtain in field exposures with cultured populations. An exposure in the period April 9–15, however, gave relative growth rates at depths of 1 m., 2·5 m., and 5 m. that were almost identical with rates obtained simultaneously with cultured material. The mean rate at 1 m. depth was 3·3 divisions/week. The last exposure (May 3–10) utilized cells from a late phase in the spring growth period, reduced to a low density by the addition of filtered lake water. Suspensions of these cells were also made in normal culture medium. Cells exposed in lake-water medium died during the experiment, whereas cells in culture medium remained healthy but showed relative growth rates that were approximately half the values recorded at 1 m. using normal cultures (Fig. 2).

These experiments suggest that the relative growth rates obtained for natural populations in lake water, sampled early during the spring growth phase, may be comparable with rates obtained for cultured populations. This similarity also extends to the photosynthetic behaviour of the two types of population. The inconsistent behaviour of natural populations at other times was also encountered by Lund (private communication) in previous field experiments. Some analogous observations were reported by Gran (1927) and Gaarder and Gran (1927) from field experiments with marine diatoms.

The Shape of Depth Profiles of Relative Growth Rates

Owing to the low relative growth rates obtained in winter it was not possible to estimate the variation of these rates with depth with any accuracy. Depth

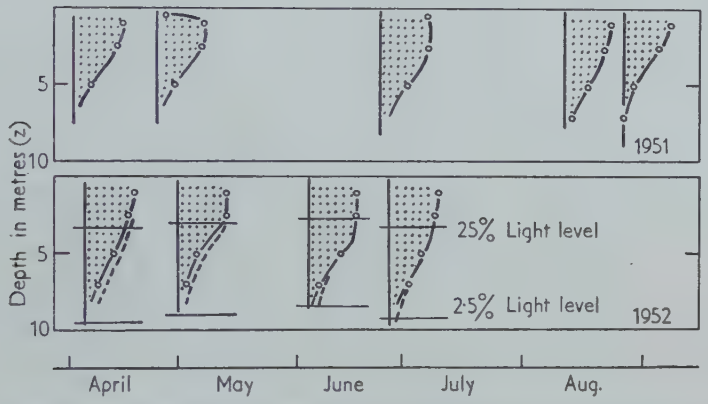

Fig. 4. Depth profiles (stippled) of relative growth rates recorded in cultures of *Asterionella* suspended in Windermere North Basin during 1951 and 1952. The maximum width of each profile is represented upon a common scale. Broken lines show integral profiles for photosynthesis calculated as described on p. 336. For each experiment in 1952 the depths are indicated at which the subsurface light intensity, expressed as the energy flux in the spectral region 400–700 mμ, is reduced to 25 and 2·5 per cent.

profiles of rates for *Asterionella* recorded in other seasons, during 1951 and 1952, are represented in Fig. 4. Each profile is plotted with the 1-m. value taken as unit width; absolute values can be obtained from Figs. 1 and 2. Temperature differences between the depths concerned were small ($< 3°$ C.) and would have little influence upon the shapes of the profiles.

The profiles shown in Fig. 4 exhibit a marked effect of light-saturation near the surface, as shown by comparison with the exponential nature of light diminution with depth. Since the determinations were fairly widely spaced in the water-column it is not possible to determine the lower limit of the profiles with any accuracy. In general it appears that little growth could occur below a depth (approx. 7 m. in Windermere) at which the intensity of photosynthetically available radiation (spectral region 400–700 mμ.) was less than about 5 per cent. of the subsurface intensity. Both as regards light-saturation behaviour and lower depth limitation, the results are comparable with the

more extensive data of Lund (1949, fig. 7) for another 'strain' of the same species in the same lake basin.

Since few measurements were made at 0·5 m. depth the experiments give little information on the possible significance of 'subsurface inhibition' behaviour, such as was found for the photosynthesis of *Asterionella* (Talling, in prep.). Such inhibition of growth was recorded by Whipple (1896) for freshwater phytoplankton at depths of less than 6 in. Inhibition of growth at 0·5 m. is represented on Fig. 4 for one profile in April 1951 and was associated with many dead cells. It is, however, possible that death resulted from other chance causes such as inadequate cleaning of the bottles. Such inhibition was never appreciable in the more numerous experiments of Lund (1949). If Lund's data are accepted as typical, it is possible that with the present techniques photosynthetic rates were more susceptible to subsurface inhibition than were relative growth rates over a prolonged period.

It is possible to regard the depth profiles shown in Fig. 4 as integrating photosynthetic rates, as recorded in short-term experiments, over a more prolonged period, with a small difference due to respiration losses. It is also possible that modifications such as 'adaptation' phenomena may occur with cells at various depths, which would distort the simple picture of time integration outlined above. The relation between time integrals of photosynthesis and the observed relative growth rates was tested as follows. Continuous radiation records during 1952 were used to compute, for each experimental period, frequency histograms of incident light intensities in various intensity ranges. These ranges were chosen in relation to a photosynthetic parameter, I_K, adopted to define the onset of light saturation of photosynthesis in relation to light intensity. The calculation of this parameter, the factors controlling its magnitude for *Asterionella*, and its use in the computation of photosynthesis-depth profiles, will be discussed elsewhere (Talling, in prep.). The values of I_K used here were derived from this data in relation to the relevant lake temperatures taken at 5 m. depth. The intensity ranges lay between the values 0·5 I_K, I_K, 2 I_K, 4 I_K, 8 I_K, 16 I_K, 32 I_K, and 64 I_K. A photosynthesis–depth profile was calculated for each intensity range, using data on vertical extinction coefficients (defining light penetration in the lake water) to be published elsewhere. Surface inhibition effects upon photosynthesis were neglected. Depth profiles for the various surface intensities were then combined additatively, allowing for the relative frequency of each intensity interval. The resulting profiles represent time-integrals of photosynthesis at various depths and are indicated by broken lines in Fig. 4. The maximum width of each calculated profile is shown equal to that of the corresponding profile of relative growth rates. In general form the calculated profiles are similar to those obtained directly for relative growth rates, but tend to extend slightly deeper into the water-column. The latter behaviour would be expected in view of respiration losses slightly reducing the calculated photosynthesis–depth profiles as represented on the figure. The available data therefore support the conclusion that, allowing for respiration losses, the

recorded profiles of relative growth rates represent a reasonably good integration of the behaviour recorded in short-term photosynthesis experiments.

Qualitative support for this conclusion is afforded by the comparison of a depth profile of relative growth rate with two depth profiles of photosynthesis (Fig. 5), all profiles being obtained within a short period (April 29–May 10, 1952). Light penetration in the lake water during this period was essentially

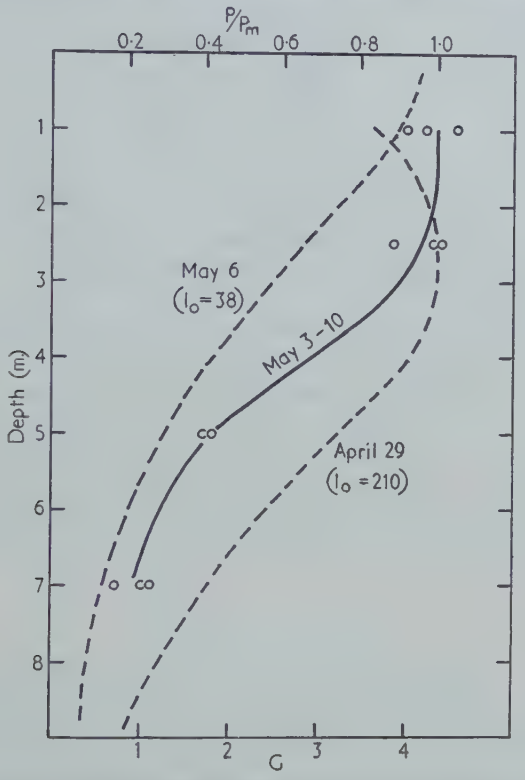

Fig. 5. Depth profiles of relative growth rates (solid line, experimental points indicated by circles) and of photosynthetic rates (broken lines) of *Asterionella* measured in Windermere in the period April 29–May 10, 1952. Photosynthetic rates are given, for each experiment, as fractions (P/P_m) of the maximum rate; relative growth rates (G) as divisions per week; and the mean surface light intensities (I_o) during the photosynthesis experiments in kiloerg/cm.^{2} sec. (spectral region 400–700 mμ).

constant, as shown by routine measurements. The growth profile is intermediate in shape between the two photosynthesis profiles, which were recorded under sunny (high surface illumination) and overcast (low surface illumination) conditions respectively.

Depth profiles of relative growth rates recorded for *Fragilaria* are shown in Fig. 6; they are obviously very similar to the profiles obtained simultaneously with *Asterionella*. This similarity also extends to the shape of corresponding profiles for *Tabellaria*, although the low relative growth rates characteristic of this species render interpretation more difficult.

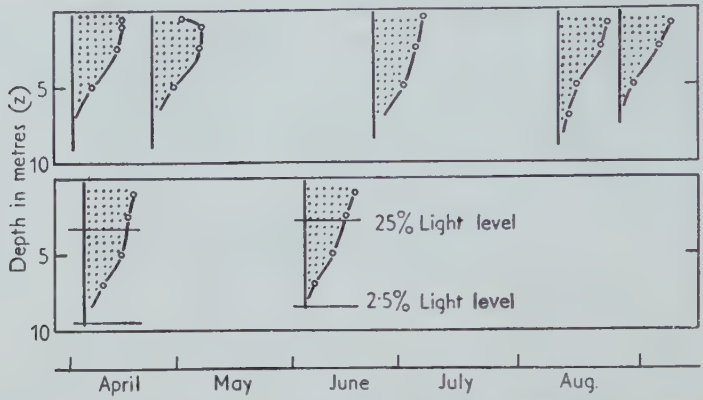

Fig. 6. Depth profiles (stippled) of relative growth rates recorded in cultures of *Fragilaria* suspended in Windermere North Basin during 1951 and 1952. Representation as in Fig. 4.

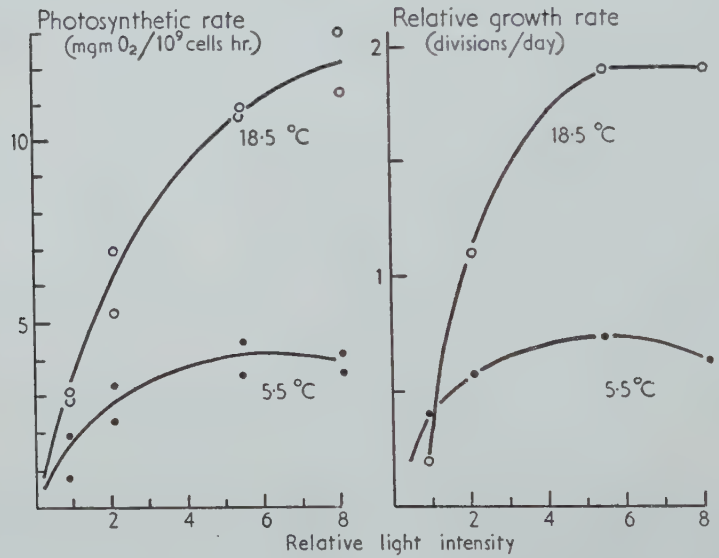

Fig. 7. Comparison of the variation, recorded under laboratory conditions, of the photosynthetic rate and the relative growth rate of cultured *Asterionella* populations in relation to light intensity and temperature. Each relative growth rate indicated is derived as a mean from two duplicate cultures.

Direct Comparison of Photosynthetic and Relative Growth Rates

Comparisons of photosynthetic and relative growth rates derived from field experiments were necessarily somewhat indirect in nature. A more direct comparison under laboratory conditions, in which photosynthetic and relative growth rates of *Asterionella* were measured at two temperatures over a range of light intensities, is illustrated in Fig. 7. Corresponding curves relating to

photosynthetic and relative growth rates are essentially similar in form except for deviations at low light intensities. The latter may arise from respiration losses and the errors involved in measuring small relative growth rates at low light intensities. However, a considerable parallelism exists between the behaviour of photosynthetic and relative growth rates in relation to light intensity and temperature. Differential behaviour in relation to light saturation, as described by Myers (1946) for *Chlorella pyrenoidosa*, is not shown to any marked extent. The parallelism encountered in the present experimental conditions need not imply a primary control of relative growth rate by factors intrinsic to the photosynthetic mechanism.

The data summarized in Fig. 7 suggest that an approximate inter-conversion of photosynthetic and relative growth rates is possible, over prolonged periods, under conditions in which respiration losses are small in comparison with photosynthesis. The mean inter-conversion is approximately

$$10 \text{ mg. } O_2/10^9 \text{ cells. hr.} = 1{\cdot}6 \text{ divisions/day}$$

and is consistent with other independent determinations, under conditions of light saturation, of photosynthetic and relative growth rates at temperatures of about 16–18° C. The conversion value given corresponds to a photosynthetic quotient of unity in conjunction with a carbon content of 56 μg./10^6 cells. Lund (1950), on the basis of mean C/N ratios, estimated the probable carbon content of *Asterionella* cells (of similar cell size) as 40–110 μg./10^6 cells. The present evidence, which is, however, approximate, suggests a carbon content near the lower limit of this range.

Summarizing Discussion

Lund (1949) has shown how field experiments of the present type can illustrate the effect of seasonal changes in light and temperature upon the growth of a plankton diatom. The observations described here extend the study to two additional species, and a quantitative interpretation of the results is attempted in relation to photosynthetic behaviour and to growth under laboratory conditions.

The comparative behaviour of the three species, under the present experimental conditions (which may not be completely representative of behaviour in other media or with different methods of exposure), reveals few differences of possible significance in relation to the varied natural periodicities of the species. The only outstanding specific peculiarity is the consistently low relative growth rates recorded for the *Tabellaria* species. Similarity of form in the depth profiles of relative growth rates suggests that the onset of light saturation of photosynthesis occurs at similar intensities in the three species. As regards *Asterionella* and *Fragilaria*, such similarity has been demonstrated experimentally and may well extend to a considerable variety of phytoplankton organisms (Talling, in prep.).

Various other features suggest a close parallelism between photosynthetic

behaviour and the relative growth rates recorded. Thus the shape of depth profiles of relative growth rates recorded for *Asterionella* is in good agreement with corresponding depth profiles of photosynthesis, both calculated (Fig. 4) and experimentally determined (Fig. 5). The parallelism is also shown (Fig. 7) in a direct comparison of variation in photosynthetic and relative growth rates under laboratory conditions. It is noteworthy that the maximum (light-saturated) relative growth rates so obtained, of the order of 2 divisions/day, are among the more rapid recorded for unicellular algae. The lower maximum rates recorded from field exposures can be interpreted in terms of the limitations imposed by varying temperature and daylength in the lake habitat.

Acknowledgements

The work described here was carried out at the Windermere Laboratory of the Freshwater Biological Association. I am indebted to the Director and staff of the Association for the excellent facilities made available to me. The investigation developed from suggestions originally made by Dr. J. W. G. Lund, and my thanks are due to both him and Dr. C. H. Mortimer for much stimulating advice and criticism. The work was aided by financial support from the Department of Scientific and Industrial Research and from a scholarship of the University of Leeds.

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The Ovule and 'Seed' of *Araucaria Bidwillii* with Discussion of the Taxonomy of the Genus

III. Anatomy of Multi-ovulate Cone Scales

BY

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With Plates VIII and IX

Abstract

This paper deals with the anatomy of two- and three-ovulate cone scales which are found occasionally in the cones of *A. Bidwillii*. The manner in which each ovule receives its vascular supply from bundles lateral to the ovule is discussed, together with a hypothetically primitive type of araucarian cone scale with three ovules possibly resembling the fossil *Schizolepis*. The interpretation of the vascular system of the scales as given in this paper is a correction of that presented in the first paper of this series by the same authors.

Introduction

THIS paper constitutes essentially an appendix to the authors' recently published studies (1948; 1952), Part I of which included a description of the anatomy of normal uniovulate cone scales. The material for the study of normal cone scales and also for this study of unusual types was obtained through the kindness of Dr. Leo F. Hadsall. During the course of collections made over several years from trees of *A. Bidwillii* planted in Kearney Park, near Fresno, California, Dr. Hadsall noted on certain trees the frequent occurrence of several two-ovulate cone scales near the base of many of the cones. Mature bract-scale units bearing two seeds were also found. Furthermore, one three-ovulate scale was found and several units bearing three aborted seeds. The maximum frequency of such unusual scales in 53 cones examined by Dr. Hadsall was found to be 14 scales per cone, the minimum frequency, 1.

In an earlier paper (Pt. I) by the present authors (1948) an interpretation of the araucarian seed-scale organization and evolution was suggested. In the light of subsequent evidence from the anatomy of two- and three-ovulate scales, this interpretation now appears inaccurate, and further discussion of the problem is given in the present paper.

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The number of ovules per cone scale in the conifers varies in different families and genera from one to many and the ovules vary in position and orientation on the scale. The larger number of ovules occurs in families commonly believed advanced, the Cupressaceae and Taxodiaceae. The solitary ovule is found chiefly in families generally recognized as possessing primitive characters, the Araucariaceae and Podocarpaceae. The uniovulate condition has by some students been looked upon as advanced (although in families primitive in other characters) and as derived from a two- or three-ovulate condition. Florin (1951) has recently described what he believes to be the fundamental organization and evolution of the seed-scale structure in various families of conifers, including the Araucariaceae. His opinion is based upon his extensive researches in the evolution of reproductive structures in the cordaites and early fossil conifers (1944).

A more recent paper of particular interest with respect to the general evolution of the genus *Araucaria* is that of Calder (1953) on the Tertiary fossil *Araucaria mirabilis* (Speg.) Windhausen (*Proaraucaria mirabilis*) from the Cerro Cuadrado petrified forest of Patagonia. Miss Calder's careful anatomical work has made it possible for her to demonstrate the vascular anatomy of the bract-scale units of this fossil araucarian. These closely resemble the scales of *A. Bidwillii* in the important primitive character of independent origin from the cone axis of bract and seed-scale vascular supply. The cone scales were as thick, woody, and widely winged as those of *A. Bidwillii*, though it was impossible to learn whether the seed was shed from the scale at maturity. The manner in which the ovule received its vascular supply was too obscure for demonstration, though it was evident that the 'ligule' was strongly vasculated. From her study Miss Calder concludes that *A. mirabilis* represents a fossil member of the recently erected section *Bunya* (Wilde and Eames, 1952) of which *A. Bidwillii* is the only known living representative.

Anatomy of Two-ovulate Bract-scale Units

The normal bract-scale unit of *Araucaria Bidwillii* is composed of a large, heavy, woody-winged bract with adnate ovuliferous[1] scale free only at the tip, and a single, median, anatropous ovule fused with the ovuliferous scale for its entire length. Near the base of some of the cones collected from trees in Fresno, California, were found units with distally two-parted sterile ovuliferous scales. In a region transitional between basal sterile scales and normal one-seeded scales, similar two-parted scales bore two ovules (Pl. VIII, Figs. 1–4; Pl. IX, Figs. 9, 11). The degree of separation of the two tips of the scale varies, the sinus between them being deep or shallow. Likewise the distance

[1] It seems advisable to discontinue the use of the term 'fertile scale' as it is applied to the scale upon which the ovule is attached in modern conifers and to use instead either 'ovuliferous scale' or 'seed-scale' for this structure. The term 'fertile scale' is now confusing and inappropriate since, in the light of our present knowledge, this structure represents in large part one or more sterile scales of the primitive axillary shoot-complex of cordaites and early fossil conifers which became phylogenetically fused with the megasporophyll.

of the ovules from one another varies (Pl. VIII, Figs. 1, 4; Pl. IX, Figs. 9, 11, 13). In young scales the ovules frequently lie several millimetres apart, but they may touch, with integuments confluent (Pl. VIII, Figs. 1–4). When the ovules mature, the seeds may be entirely separate or fused together (Pl. IX, Figs. 9, 11, 13). Close-lying ovules were usually found associated with single-tipped scales. Mitra (1927) has found similar scales in other species of *Araucaria*, as well as scales with two 'ligules' completely separate from one another. An unusual position for the two ovules, apparently not found by Mitra in other species, was observed on some of the bract-scale units. One ovule is medianly placed, the other at one side, suggesting the loss of a third ovule on the other side (Pl. VIII, Fig. 5). In such placement the free tips of the scales (where present) are on a line with the ovules. In these scales the central ovule and scale tip are frequently the better developed. Towards the apex of the cones some complete bract-scale units were found laterally fused together (Pl. IX, Fig. 10).

Transverse, free-hand sections of young two-ovulate bract-scale units were cut for the purpose of studying the vascular anatomy and comparing it with that of the typical uniovulate units (Eames, 1913; Wilde and Eames, 1948). A dissection was also made of older units by teasing away the tissues surrounding the vascular bundles of the seed-scale (Pl. VIII, Figs. 7, 8). At the base of the two-ovulate bract-scale units, two series of bundles are present as in typical scales, the lower series supplying the adnate bract; the inverted upper series, the ovuliferous scale. The arrangement of bundles in this inverted series was most significant in those scales in which the two ovules were rather widely spaced. As in normal scales, several outer lateral bundles, frequently anastomosing, run the length of the ovules close to the stony layer of the integument, and bend into the chalazal region where ovular branches are given off from a plexus (Pl. VIII, Fig. 8, *pl*) of anastomosing bundles which ultimately continue outward into the free scale tips (Pl. VIII, Fig. 7). Such a plexus is present in the chalazal region of each ovule in the two-ovulate units (Pl. VIII, Fig. 8). It has been noted in one-ovulate scales (Wilde and Eames, 1948) that scale bundles dorsal to the ovule are frequently included in the stony 'integumental' layer. These bundles, sometimes weak and inconspicuous, may terminate within this 'integumental' layer or, after giving off branches to the ovule, may continue a short distance beyond the chalazal region with apparently no connexion with the above-mentioned chalazal plexus formed from the lateral bundles. In two-ovulate scales these bundles dorsal to the ovule may be almost lacking, or, if present, are conspicuous only for a short distance beyond the micropylar region, apparently then becoming attenuate and lost (Pl. VIII, Fig. 8, *db*). In scales with rather widely spaced ovules a strong group of bundles is present between the ovules (Pl. VIII, Figs. 7, 8). The two or three bundles of the group that lie contiguous to each ovule bend into their respective chalazal regions and there contribute to a chalazal plexus (Pl. VIII, Fig. 8, *pl*). Numerous other bundles, however, lying between the ovules, have a characteristic position, possibly suggestive of a third ovular

supply. In transverse section they may lie in two oblique radial rows which meet near the ventral surface of the scale or in a single strongly convex line. The bundles within each row lie close together, many anastomosing into strong radial sheets of vascular tissue. In transverse section they often appear irregularly oriented, as do also the bundles on the outer sides of each ovule. Sometimes groups of bundles are arranged in a circle with xylem towards the periphery; other bundles seem to be concentric. These bundles do not contribute branches to the two ovules but, beyond the chalazal level of the scale, continue outward in a convex line (as seen in cross-section) to the very edge of the sinus between the two free tips of the ovuliferous scale. In several species of *Araucaria*, Mitra (1927) noted the disposition of the scale bundles in three groups in transitional scales with two free 'ligular' tips, but found the median group usually poorly developed.

This independent central series of bundles is absent in those scales in which the integuments of the ovules are confluent and is also missing in those two-ovulate scales in which apparently one median and one lateral ovule are present. In the few scales of this type examined, one (ultimately forked) or two bundles only occur between the ovules, each contributing to an ovular plexus in the chalazal region.

Three-ovulate Bract-scale Units

A single, young, two-parted bract-scale unit was found bearing three ovules (Pl. VIII, Fig. 6). One ovule is separate, the other two lie close together but with two distinct micropyles. Some bract-scale units in nearly mature cones show traces of a third ovule, and a few mature units were found with three aborted seeds.

Transverse sections of the one young bract-scale unit bearing three ovules were cut in order to determine the course of the scale bundles. Dorsal scale bundles seem to be entirely lacking. Two of the three ovules lie so close together that there is no stony integumental layer between them. Only a single lateral bundle lies in the shallow dorsal sinus separating them. The third ovule is entirely separate from the other two, except at its broadest part, where its integument just touches that of the median ovule. Several lateral bundles are present between this separate ovule and its neighbour. Nearly all these lateral bundles are tied into the plexus of either one or the other of the adjacent ovules with the exception of four small bundles which continue entirely free from the other bundles into the upper part of the scale. This, of course, resembles the arrangement of bundles in the two-ovulate bract-scale units, except that in these, more free bundles are present between the adjacent ovules than were found in the three-ovulate unit.

Discussion

The type of vasculation in two-ovulate cone scales, together with the occurrence of some three-ovulate scales, supplies evidence in support of the hypothesis that araucarian cone scales primitively bore three ovules which were

ultimately reduced to one. It is probable that the centrally placed vascular tissue lying between the two ovules was associated with a lost ovule. Does the single surviving ovule in typical scales represent a median ovule, a lateral one, or two fused lateral ovules? It was suggested by Wilde and Eames (1948) that the surviving ovule may be a lateral one. This view was based upon the fact that, in normal one-seeded scales, lateral bundles, rather than bundles dorsal to the ovule, contribute the main ovular supply. But the supply to the ovules is similarly derived from lateral bundles in the two- and three-ovulate scales. Moreover, in these scales the dorsal series of bundles is even more weakly developed than that in uniovulate units. Another interpretation, therefore, of this unusual bundle arrangement is necessary. Florin (1951) also has expressed doubt concerning the present authors' interpretation that the single surviving ovule of normal scales represents a lateral one. He writes (p. 361): 'Normally, the rudimentary floral axis carries in my opinion only one median sporophyll and one sterile scale on the side facing the cone-axis, but in abnormal cases the median pairs of scales may be completely suppressed, and two transverse pairs, one fertile and one sterile, developed.'

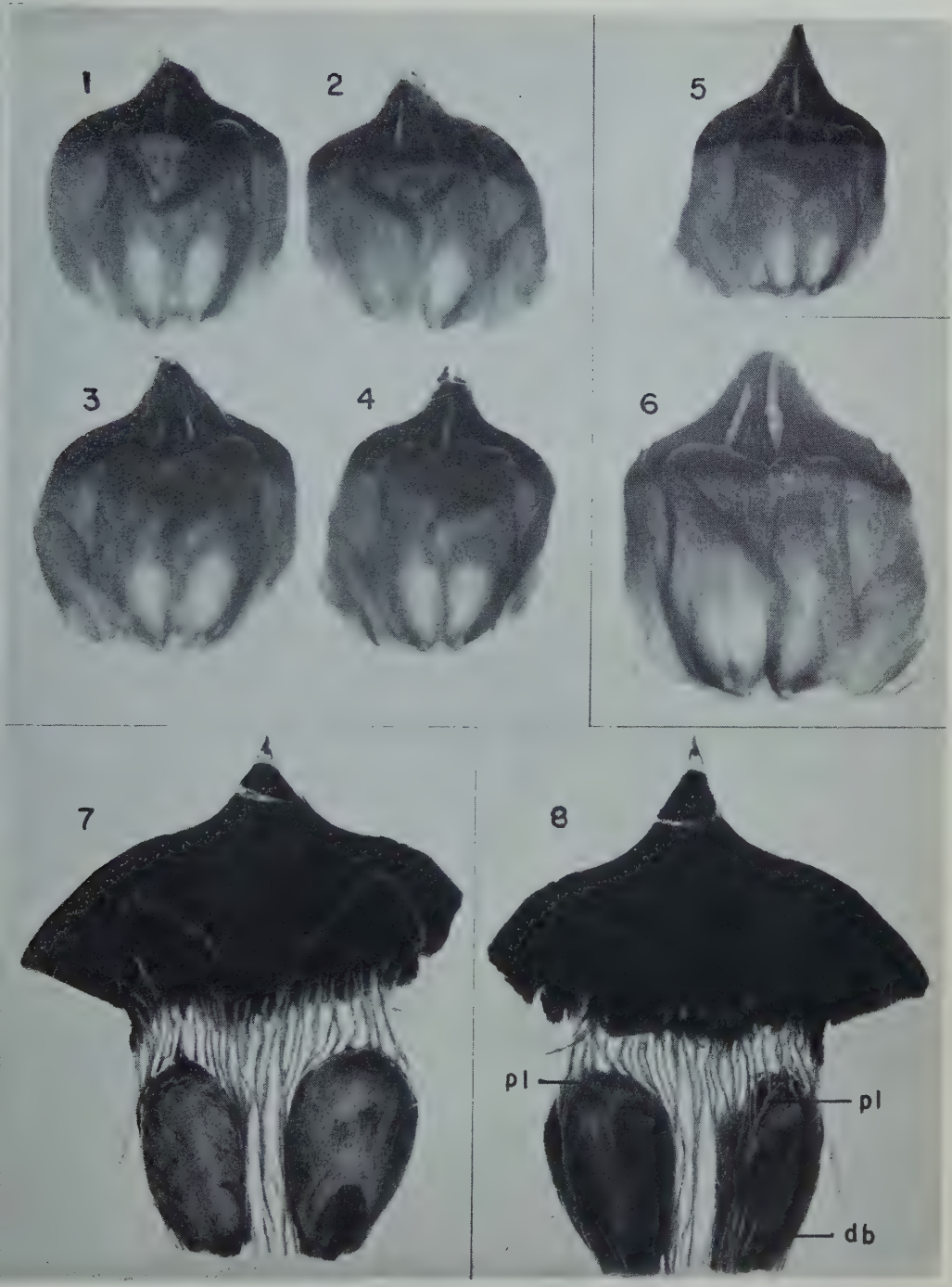
Mitra (1927) clearly recognized that the uniovulate condition is probably a 'secondary' or 'derived' feature. He states (p. 470): 'We may suppose that in a scale with three potential ovules the median one alone develops; the scale of *Cunninghamia* and a *Protodammara* may thus represent primitive types, in which all three ovules are developed.' But he further suggests (p. 464): 'The presence of two "ligules" and their ultimate fusion into one in the transitional sterile scale, suggest that we may have here the vestiges of two lateral sporangia which have become united into a median solitary one.'

In two-seeded araucarian cone scales the apparent association of an ovuliferous scale with each ovule could not clearly be understood by Mitra in 1927 for lack of the fossil evidence we have today. Florin (1944) has shown that during the series of reductions, fusions, and flattenings that took place in the primitively radially constructed fertile shoot axillary to the bract, each megasporophyll (often recurved), bearing a terminal ovule, became fused with a sterile scale of the shoot in such a way that the ovule appears to lie on the ventral surface of the leafy appendage. Each ovule, therefore, early became associated with a sterile scale which, in present-day conifers, is clearly represented by the distal portion of the ovuliferous scale on the surface of which the ovule now appears to be seated. In some early Mesozoic seed-scale complexes, however, including those of *Schizolepis*, a broad, flat ridge is visible running from the base of the complex up to the ovule attachment on the sterile scales. These ridges represent in *Schizolepis* three proximal megasporophylls fused each with three distal underlying sterile scales of the reduced and flattened shoot. Both sterile scales and megasporophylls respectively were fused to one another at the base of the complex. The appearance of two- and three-parted ovuliferous scales associated with two and three ovules in *Araucaria* is thus reminiscent of the three-parted seed-scale complex of *Schizolepis*.

Florin (1951, diagrams, pp. 363, 346) believes the basic organization of both the araucarian seed-scale complex and that of *Schizolepis* consisted originally of an axillary shoot bearing two proximal pairs of decussately arranged megasporophylls with terminal ovules and two pairs of distal sterile scales. The seed-scale complex of the Mesozoic *Schizolepis* therefore had presumably lost one megasporophyll and one sterile scale on the side of the fertile shoot next to the bract, while normal araucarian seed-scale complexes had in addition lost the two laterally placed megasporophylls with their fused sterile scales.

Granting that the single remaining ovule on normal araucarian cone scales probably represents the survival of a median one out of a possible three (or four), the manner in which that ovule receives its vascular supply is unusual. The interpretation of the entire vascular system of araucarian bract-scale units, both one- and two-seeded, even in the light of our new understanding of their morphology, is still difficult. It may be supposed that the weak series of bundles dorsal to the ovule represents the vascular supply of the megasporophyll (probably fused with some bundles of the underlying sterile scale). This series must have run originally directly into the chalazal region of the ovule. Fusions between bract, sterile scales, and megasporophylls brought the bundles of these organs into close proximity. It is a well-known fact that adjacent bundles tend to become phylogenetically fused. The bract (probably the last organ in the araucarian bract-scale unit to become coalesced) retained its identity with respect to its bundle supply in *A. Bidwillii* alone of all the living species so far studied, and in *A. mirabilis*, a Mesozoic species. Fusions, however, between bundles of megasporophyll and sterile scale must have taken place phylogenetically much earlier than similar fusions involving the bract. The strong bundles of the scale lying lateral to the ovule may represent bundles which belonged originally only to that sterile scale which early became fused with the megasporophyll. If this be true, those bundles contiguous to the ovule still show traces of fusion with megasporophyll bundles in the plexus of bundles in the chalazal region of the seed. The strong development of the large seed downward into the bract-scale unit has undoubtedly not only given the seed the appearance of being sunken in the scale but has 'crowded out' the bundles dorsal to the ovule, that is, megasporophyll bundles originally supplying the ovule. This 'crowding out' of what may have once been the ovular supply through the megasporophyll could finally have resulted in the taking over of the function of main ovular supply by the lateral sterile scale bundles tied into the plexus.[1] Such changes in the vascular system must have taken place in each member of the three-parted seed-scale complex before reduction to a single ovule. Each of the ovules on the two- and three-ovulate araucarian scales might then be expected to possess such a characteristic vascular supply. Normally, the median ovule of the three may have been

[1] I should like to express my appreciation for the privilege and pleasure of discussing this interpretation with Professor Florin during his visit to Washington in September 1948. At that time he agreed that this seemed to be the only logical interpretation for such bundle behaviour. M. H. W.



M. H. WILDE *and* A. J. EAMES

M. H. WILDE *and* A. J. EAMES

retained; on some two-seeded scales, lateral ovules may be present, the centrally placed series of bundles between them representing the vestiges of the median ovule and sterile scale. On other two-seeded scales a median and one lateral ovule seem to be present. The frequent occurrence in *A. Bidwillii* of such scales in otherwise normal cones suggests that these are not mere monstrosities but have phylogenetic significance. The frequency of two-seeded scales in several living species of *Araucaria* seems to indicate that reduction to a single ovule took place in the not too distant geological past.

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EXPLANATION OF PLATES

Illustrating Associate Professor M. H. Wilde and Professor A. J. Eames's article on 'The Ovule and "Seed" of *Araucaria Bidwillii*. III'

PLATE VIII

Figs. 1–8. Two- and three-ovulate bract-scale units of *A. Bidwillii*.

Figs. 1–4. Two-ovulate (except Fig. 2) bract-scale units, about fertilization stage, showing relative distance of two ovules from one another and relative depth of sinus in the two-parted ovuliferous scales. (×4/5.)

Fig. 5. Two-ovulate bract-scale unit showing trace of a third undeveloped ovule. (×4/5.)

Fig. 6. Three-ovulate bract-scale unit, about fertilization stage. (×7/5.)

Fig. 7. Ventral view of dissected bract-scale unit (older) showing position of scale bundles. Bract bundles removed. (×7/5.)

Fig. 8. Dorsal view of dissected bract-scale unit (older) showing strong lateral scale bundles anastamosing in a chalazal plexus (*pl*) and weak dorsal scale bundles (*db*). Several bract bundles just visible in cut portion of unit. (×7/5.)

PLATE IX

Figs. 9–13. Mature bract-scale units and 'seeds' of *A. Bidwillii*, photographed by Dr. Leo F. Hadsall. (×4/5.)

Fig. 9. Mature bract-scale unit showing two partly fused 'seeds'.

Fig. 10. Two mature complete bract-scale units, from apex of cone, laterally fused.

Fig. 11. Mature bract-scale unit showing two separate 'seeds'.

Fig. 12. Single normal 'seed'.

Fig. 13. Two fused 'seeds' removed from bract-scale unit.

Studies in the Physiology of Parasitism

XIX. On the Killing of Plant Cells by Enzymes from *Botrytis cinerea* and *Bacterium aroideae*

BY

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With Plates X–XII and five Figures in the Text

Abstract

1. Enzyme preparations were obtained from culture filtrates of the soft-rot pathogens *Botrytis cinerea* Pers. and *Bacterium aroideae* (Townsend) Stapp grown in simple synthetic nutrient media. Crude culture filtrates and preparations purified by acetone-precipitation and dialysis had three characteristic properties. They (i) decreased viscosity of pectin and pectate solutions, (ii) macerated parenchymatous tissues of higher plants, and (iii) killed cells of tissues so macerated. A parallelism was demonstrated between activity estimated by these three criteria.

2. *B. cinerea* enzyme preparations were active from about pH 3·5 to pH 6·0, activity decreasing rapidly from pH 6·0 to nearly nil at pH 8·0. Conversely *B. aroideae* was most active above pH 8·0, activity decreasing progressively to nearly nil at pH 5·5.

3. Both enzymes lost much activity on prolonged dialysis against distilled water and this was not recovered on readdition of dialysed salts. On dialysis against certain salts or salt mixtures reduced or negligible losses occurred.

4. Plasmolysing concentrations of salts or non-electrolytes greatly retarded the killing action of the enzyme preparations, the effect being out of all proportion to that on maceration or on rate of pectin degradation.

5. Protoplasts were isolated in the plasmolysed condition from certain tissues. These were resistant to toxicity in similar manner to those inside the tissue.

A. Introduction

An examination of the necrotic areas of plant tissues infected by a soft-rot pathogen reveals that the affected cells are separated from one another and are dead. In studying the physiology of the process, Brown (1915)

[1] Now at the School of Agriculture, Cambridge.

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prepared an extract from young germ-tubes of *Botrytis cinerea* which caused a rapid maceration of discs cut from certain tissues and subsequent death of the cells. He was unable to separate the macerating activity of his extract from its lethal activity by any process of differential deactivation, and postulated two alternative ways of explaining the relationship between the phenomena: (1) that the cells were macerated and killed by the same substance (or group of substances), or (2) that the cell-walls were rendered permeable by the macerating substance to a separate, presumably colloidal, toxin. If the former postulate were correct, the interpretation would be that the macerating substance was directly toxic or that it caused death indirectly as a result of action on the cell-wall.

The macerating action was attributed to an enzyme which caused breakdown of the middle lamella and which was originally referred to as a cytase and later as a pectinase, but which is now usually termed protopectinase. Since Brown's paper was published, many workers have prepared tissue-macerating enzymes, but attention has not been paid to any toxic action of these preparations.

Thatcher (1942) found that *Botrytis cinerea* and *Sclerotinia sclerotiorum*, when inoculated into celery tissue, caused a fourfold increase in permeability of cells just beyond the discoloured and necrotic zone. He further claimed that permeability changes could be detected inches away from such lesions and concluded that some factor other than pectinase contributed to the phenomenon of action in advance shown by soft-rot pathogens. He considered that this factor might be a necessary precursor to pectinase activity but made no suggestion as to its nature.

The object of the present investigation was to examine further the relation between the macerating and lethal phenomena in soft-rot parasitism. Attention was restricted to the two organisms *Botrytis cinerea* Pers. and *Bacterium aroideae* (Townsend) Stapp and two lines of approach to the problem were followed.

1. A comparative study of pectic enzymes produced in culture filtrates by the two organisms was made on pectin solutions, and on tissue discs with respect to maceration of the tissue and killing of the cells. Variations in enzymic activity were brought about by altering conditions of pH, by dialysis, and by the action of salts.

2. Protoplasts were plasmolysed and liberated from certain tissues. By thus removing them from the cell-wall barrier it was hoped that it would be possible, in conjunction with suitable deactivation tests, to prove directly the presence or absence of the postulated colloidal toxin.

B. Materials and Methods

The organisms used were a densely-sporing strain of *Botrytis cinerea* and Dowson's Strain 66 of *Bacterium aroideae*, both maintained in the culture collection of the Imperial College of Science and Technology. Active enzyme

preparations were obtained by shallow culture technique. Spores of *Botrytis cinerea* were heavily inoculated into layers of the following medium: glucose, 10 g.; ammonium tartrate, 5 g.; KH_2PO_4, 1 g.; $MgSO_4.7H_2O$, 0·5 g.; distilled water, 1 litre. For *Bacterium aroideae* the same medium was used but with 10 g. $CaCO_3$ added per litre in order to neutralize acids produced by this organism.

The enzymes contained in the crude culture filtrates after 4 to 6 days' growth were concentrated and partly purified by acetone precipitation and dialysis. Two volumes of acetone were added to one of the crude preparation, both having been precooled to 5° C. *Botrytis* extract when so treated gave a finely granular precipitate, much of which was a magnesium ammonium phosphate. A coagulum which floated was also produced; this had no activity and was discarded. Supernatant liquid was decanted off after standing for some hours in the cold room and the precipitate was collected by centrifugation. It was extracted twice in distilled water or dilute phosphate buffer at pH 5·6. Some 30–60 per cent. of the total activity survived the precipitation. Residual acetone was removed under reduced pressure and the concentrated extract stored under toluene at 5° C.

The crude bacterial preparation when treated with acetone gave a copious flocculent precipitate which settled fairly rapidly. It was extracted in phosphate buffer at pH 8. Residual acetone was removed before extraction, since sufficient was included in the voluminous precipitate to keep the enzymes out of solution. The concentrated extract was centrifuged free of bacteria and stored under toluene at 5° C.

Some of the preparations so obtained were further purified by dialysis. The *Botrytis* concentrates were dialysed against a solution containing: 0·025 M. NaCl, 0·001 M. $MgSO_4$, and 0·004 M. phosphate buffer pH 5·6, and the bacterial solutions against: 0·025 M. NaCl, 0·001 M. $MgSO_4$, 0·01 M. $CaCl_2$, and NaOH to pH 8·0. These salt mixtures were used since preliminary experiments showed loss of activity of crude filtrates when dialysed against distilled water.

The macerating activity of preparations was determined in some cases by Brown's original method (*loc. cit.*) in which the end-point was taken when tissue discs of standard thickness gave no perceptible resistance when gently pulled apart; alternatively the end-point was taken when the discs broke in pieces on being lifted from the enzymic solution by forceps. The latter method was particularly satisfactory for cucumber tissue.

Toxicity was estimated by placing discs of tissue into a plasmolysing agent containing the vital stain neutral red. By this means it was possible to observe living protoplasts directly as red spots in a disc of tissue, and discs from various solutions could be compared together in one view. Discs were transferred from enzymic solutions to a mixture of the following composition: Molar KNO_3, 8·5 ml.; 0·1 per cent. neutral red chloride, 1·0 ml.; phosphate buffer (pH 7·6), a few drops. This was prepared freshly for each estimation as crystals were deposited from the solution at the weakly alkaline reaction

which is optimal for absorption of the dye by the cells. The sections remained in this solution for 20 minutes, the plasmolysing agent preventing any further toxic action. Comparisons were made visually and the results were recorded by number, thus: 5, whole disc covered with red spots, 4, 3, 2, 1, gradations; 0·5, 0·1, occasional spots; 0, no spots visible. These numbers are referred to in the text as the Neutral Red Index. For permanent record the discs were rinsed free of excess stain in molar potassium nitrate, transferred to a white tile and photographed in a shallow layer of potassium nitrate solution.

In these tissue experiments it was necessary to make sure that no harmful concentration of toluene was present. Solutions stored under toluene were sampled from under the surface by pipette and left in an evaporating basin for about an hour until no smell of toluene was detectable. Tests on control solutions similarly stored under toluene showed that no toxicity remained after this treatment.

Activity on soluble pectic substances was determined by a standard viscometric method as previously described by Wood (1955).

The technique employed in obtaining and studying extracted protoplasts will be described in section D.

C. Comparative Study of Pectic Enzymes of *Botrytis cinerea* and *Bacterium aroideae*

(i) *Effect of pH on activity*

(*a*) *Tissue substrates*

The action of *Botrytis* and *Bact. aroideae* enzymes[1] was studied comparatively on potato discs over the pH range 4·0–8·6. Dialysed acetone-precipitated enzyme preparations were employed, of approximately equal activity when measured against the discs at pH 5·5 for *Botrytis* and pH 8·6 for *Bact. aroideae*, their respective optima. Buffering was by sodium citrate—NaOH (or HCl) over the pH range 4·0–6·8, and by boric acid—sodium chloride—NaOH from pH 7·6–8·6. Boric acid and sodium chloride, in the same concentration as for the alkaline buffer but without NaOH, were added to the citrate buffer; to the borate buffer was added sodium citrate at pH 6·8, i.e. at the end of its buffering range. By this means the concentration of the buffer salts was kept constant over the range of pH values investigated.

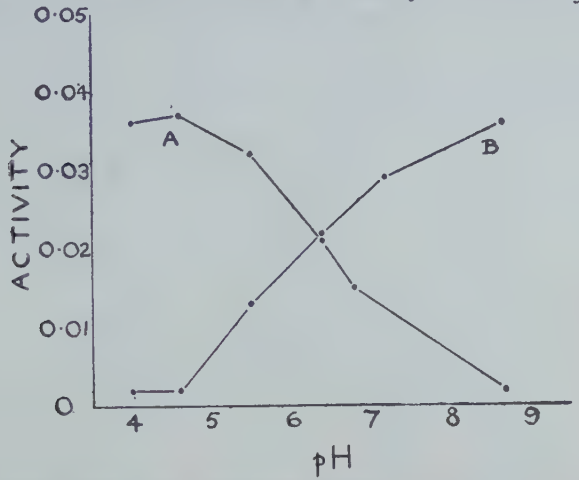
Text-fig. 1 gives the relation of the macerating activity to pH, the former being taken as the reciprocal of the macerating time in minutes. It brings out the converse behaviour of the macerating (protopectinase) activity of the enzymes from these two organisms.

The effect of pH on toxic action on the protoplasts, as followed by the Neutral Red Method, is illustrated in Pl. X, Figs. 1 and 2. The living cells, having accumulated the stain in the vacuoles, show up as black dots.

The influence of pH on rate of toxic action runs parallel to that on rate of

[1] To avoid confusion of nomenclature the pectolytic solutions used are referred to simply as *Botrytis* or *Bact. aroideae* enzyme.

maceration. At maceration time, when the discs had lost coherence, relatively few cells remained alive. In Pl. X, Fig. 1, many cells in *Botrytis* enzyme at



TEXT-FIG. 1. *Botrytis cinerea* and *Bacterium aroideae* enzymes. Relation of pH with macerating activity on discs of potato tissue. A. *B. cinerea* enzyme. B. *B. aroideae* enzyme.

$$\text{Activity} = \frac{1}{\text{maceration time (min.)}}.$$

pH 4·6 and 5·5 are dead, as are those in *Bact. aroideae* enzyme at pH 8·6, these maceration times being between 25 and 35 minutes.

On carrot tissue, with discs of 0·18 mm. thickness, results were similar (Table I). The toxic action of the preparations is shown in Pl. XI, Figs. 1 and 2.

TABLE I

Effect of pH on Maceration and Killing of Carrot Tissue by Enzymes of B. cinerea and Bact. aroideae

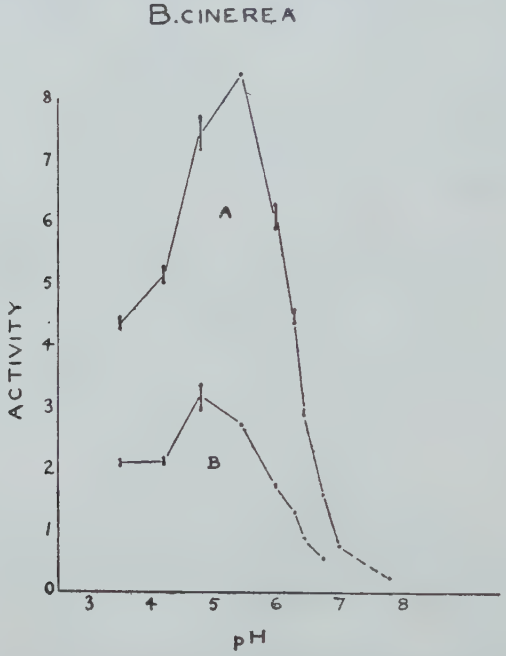
	B. cinerea.		*Bact. aroideae.*	
pH.	Maceration time (min.) (Av. of 12 discs).	N.R.* Index (at 30 min.).	Maceration time (min.) (Av. of 12 discs).	N.R.* Index (at 30 min.)
4·6	8	1	> 180	5
5·5	5	1	68	5
6·6	8	1	18	0–1
8·8	> 180	5	8	0

* Neutral Red Index, see p. 354.

At the respective optimal pH values for enzyme activity, the majority of the cells are dead; conversely, at the unfavourable pH values, the cells stain and plasmolyse normally. The typical formation of small globules which stain deeply with neutral red after plasmolysis of cells treated with *Botrytis* enzyme is well shown in Pl. XI, Fig. 1 (left). This is contrasted with Pl. XI, Fig. 2 (right), where the treatment with active *Bact. aroideae* enzyme does not result in this globulation. The characteristic differences in the cell-wall

appearance observed in potato by Talboys (1950) are shown, the cell-walls of sections treated with the bacterial enzyme at pH 8·8 being more completely broken down than in the corresponding preparation for *Botrytis* enzyme at pH 5·5.

The enzymic effects on cucumber tissue were substantially the same as on potato and carrot tissues.



TEXT-FIG. 2. *Botrytis cinerea* enzyme. Relation of pH with activity on 0·25% pectin.
A. Activity measured at 50% V.L.
B. Activity measured at 75% V.L.
} in 0·04 M. McIlvaine citrate-phosphate buffer.

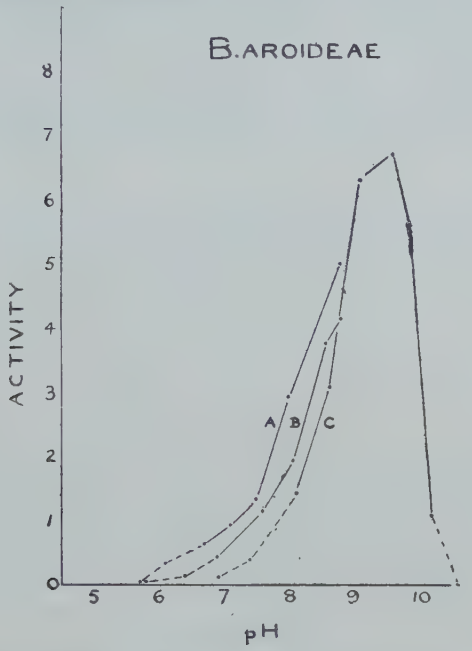
(b) Soluble pectic substances

The activity of both enzymes was studied on 0·25 per cent. lemon pectin solution.[1] Additional study was in the case of *Bact. aroideae* made on sodium pectate, prepared by de-esterification of the pectin with orange pectinesterase and neutralization with sodium hydroxide. Pectate was used here since the optimum pH for the bacterial enzyme lies beyond 9, at and beyond which value the viscosity of pectin decreases by direct action of alkali, whereas that of pectate remains stable. The solutions were appropriately buffered and rate of loss of viscosity with time was determined after the manner described by Wood (1955). The times required to give a certain percentage viscosity loss were read off from the viscosity-time curves so obtained.

Activity, as measured by the inverse of the time required to achieve a given viscosity-loss (V.L.), is plotted against pH in Text-fig. 2. The two graphs

[1] Grade 329, kindly supplied by General Foods Corporation, Birely Division, Hoboken, New Jersey; degree of methylation 74·3 per cent. (by number).

confirm previous records obtained by a variety of methods that *B. cinerea* enzyme is active in acid solution and that its activity decreases sharply at the neutral point. The optimum pH was slightly different according to the stage at which activity was measured, viz. at pH 4·8 and 5·4 in the graphs for 75% V.L. and 50% V.L. respectively. The data suggest that this difference is real, but more detailed work would be required for its interpretation.



TEXT-FIG. 3. *Bacterium aroideae* enzyme. Relation of pH with activity.

A. Crude preparation. Activity measured at 60% V.L. on 0·25% pectin in 0·01 M. phosphate buffer.

B. Acetone-precipitated preparation. Activity measured at 75% V.L. on 0·25% pectin in 0·01 M. veronal acetate buffer.

C. Ditto, on 0·25% pectate.

Activity-pH graphs for *Bact. aroideae*, for three different preparations of the enzyme, are shown in Text-fig. 3. The optimal activity on pectate was between 9·1 and 9·6, with a rapid fall on the more alkaline side to practically nil about pH 10. On pectin there was close agreement up to pH 8·6, beyond which, owing to alkaline degradation of pectin, no comparison was possible.

Text-figs. 2 and 3 show general agreement, i.e. similar optima, with the corresponding graphs in Text-fig. 1. The slope of the curves downwards from the optimum is, however, distinctly more gradual in the latter. Thus with *Bact. aroideae* enzyme, macerating activity at pH 6·4 is about 60 per cent. of that at the optimum as against some 10 per cent. when activity is measured viscometrically; and similarly for *B. cinerea* enzyme. Lack of fuller agreement is explainable, in part at least, by the non-linear relation between concentration and macerating activity, noted by Brown (1915) for *B. cinerea*

preparations, and by Wood (1955) for those of *Bact. aroideae*. A smaller decrease in activity than would be expected results from serial dilution. This is illustrated in Table II, where acetone-precipitated preparations of the enzymes, standardized to be of equal activity on pectin solution at their optimal pH values, were compared in serial dilution on potato tissue. The preparation of *Bact. aroideae* was also more active than that of *B. cinerea* in macerating the tissue. This disparity of activity increases with increasing dilution; with further dilution activity of the *Botrytis* enzyme falls off before that of *Bact. aroideae*.

It is evident from this section that there is a parallelism between enzyme activity as measured by maceration of tissue, killing of cells, and reduction of

TABLE II

Effect of Dilution on Macerating Activity of B. cinerea and Bact. aroideae Enzymes of Equal Activity on Pectin

Concentration of enzyme.	Maceration time (min.). Av. of 12 discs. *B. cinerea* (pH 5·5).	*Bact. aroideae* (pH 8·6).
1	28	21
0·5	42	23
0·25	74	30
0·125	144	45

viscosity of soluble pectic substrates. *B. cinerea* preparations possess optimal activity between pH values 4 and 6, whilst those from *Bact. aroideae* have an optimum at about pH 9. Conversely, the enzymes are inactivated in alkaline and acid conditions respectively.

(ii) *Effect of dialysis*

Preliminary tests with crude *B. cinerea* enzyme preparations showed that on thorough dialysis against distilled water much activity was lost. The technique was therefore examined more closely.

The cell used was an electrodialyser which had three compartments. The central one was narrow (0·7 cm. across) and contained 20 ml. of the solution to be dialysed. It was separated from each electrode compartment by Cellophane membranes of area 45 sq. cm., which were cut from British Cellophane Company P.T. 300 sheet Cellophane. 200-volt D.C. supply was used and the current set at a maximum of 0·3 amp. at the commencement of the dialysis by adjustment of a rheostat. After conductivity had fallen, the resistance was decreased to raise the voltage. A stream of distilled water was run past the electrodes, and the dialysis was concluded when the conductivity of the solution dropped nearly to that of distilled water.

Dialyses without the current were carried out in this apparatus by placing 50 ml. of distilled water (or other solution) in the outer compartments. This was changed at intervals, five changes in 2 days being usual. A drop of toluene was added to the enzyme preparations. Membranes were always carefully tested for leaks before use.

(*a*) *Enzyme of B. cinerea*

Table III gives the results of an electrodialysis experiment. There was a rapid removal of the cations potassium and magnesium with an associated rapid fall in pH, but later the pH rose again as the anions were removed.[1] A progressive decrease in activity took place both with regard to macerating and to toxic effects and after $3\frac{1}{2}$ hours' treatment deactivation was nearly complete. Before testing with cucumber tissue, all the preparations were brought to pH 5·6.

Restoration of the ions which had been dialysed away did not restore activity; also deactivation was not caused by the exposure to high acidity

TABLE III

Loss of Macerating and Toxic Activities by Crude B. cinerea Enzyme when subjected to Electrodialysis, as estimated on Cucumber Tissue

Duration of electro-dialysis (min.).	pH.	Ions detectable.	Maceration time (min.).	N.R. Index At 60 min.	N.R. Index At 180 min.
0	6·2	K', Mg'', SO_4'', PO_4'''	20	0·5	0
30	2·4	SO_4'' and PO_4'''	35	1–3	0·5
90	2·6	PO_4'''	100	3–5	1–3
150	3·4	PO_4'''	>150 <16 hrs.	3–5	1–5
210	4·0	None	>16 hrs.	5	4–5

which was incidental to treatment. The following figures illustrate these points:

	Maceration time.
Undialysed preparation	18 min.
Ditto, acidified to pH 2·6 with H_3PO_4	23 ,,
Electrodialysed, with or without readdition of KH_2PO_4 and $MgSO_4$ as in nutrient medium	5 hrs.

All were tested at pH 5·6 on cucumber discs and parallel results were obtained for toxic activity.

A crude preparation of *B. cinerea* enzyme was filtered through a Cellophane membrane under pressure and some of the ultrafiltrate was added to a sample of the enzyme inactivated by dialysis. Again, there was no restoration of activity.

Similar results were obtained when enzymic activity was estimated by the viscometric method. *Botrytis* enzyme was dialysed without the electric field against distilled water for 40 hours. Activity fell to one-ninth that of the undialysed control, and addition of autoclaved undialysed enzyme had no effect on restoring activity.

[1] Chemical tests were made for the ions present as constituents of the medium: sodium cobaltinitrite for potassium, magneson reagent for magnesium, barium chloride for sulphate, ammonium molybdate and benzidine for phosphate; all as in Vogel (1945).

(*b*) *Enzyme of Bact. aroideae*

Preparations of *Bact. aroideae* enzyme, like those of *B. cinerea*, lost activity when dialysed against distilled water. Against certain salts, the loss of activity was reduced. Dialyses of acetone-precipitated preparations were carried out (A) against distilled water, (B) against 0·025 M. NaCl, and (C) against 0·01 M. $CaCl_2$. After dialysis for 40 hours, 0·01 M. $CaCl_2$ was added to preparations (A) and (B), as it is known that the calcium ion is necessary for the functioning of the enzyme (Wood, 1955). The three preparations, together with an undialysed control (D), were adjusted to pH 8 with veronal-acetate buffer and assayed viscometrically. They were also assayed on cucumber discs in 0·01 M. citrate-borate buffer. The results are shown in Table IV.

TABLE IV

Effect of Dialysis on Activity of Bact. aroideae Enzyme

Preparation.	Activity on pectin. Time to 50% V.L. (min.).	Activity on cucumber discs (in 1/20 dilution). Maceration time (min.).	N.R. Index at 35 min.
A	22	100	5
B	18	100	5
C	3	20	0
D	1	15	0

The following conclusions are drawn from this section. Under conditions of prolonged dialysis against distilled water, both *B. cinerea* and *Bact. aroideae* enzymes lost most of their activity, measured either by the viscometric method or by maceration and killing of tissue discs. No recovery of activity was found after adding back dialysable solutes in various forms to the dialysed preparations. On dialysis against certain salt solutions under otherwise identical conditions loss in activity was much reduced.

(iii) *Effect of salts and other compounds*

Brown (1915) showed that various salts, if present in sufficient concentration, retarded the macerating activity of *B. cinerea* enzyme, this effect being much greater at equivalent concentrations of salts containing bivalent ions. Solutions of non-electrolytes such as sugars, of equivalent molarity, were much less active. A similar effect of salt concentration (KNO_3, $MgSO_4$) has been established in this work on the rate of hydrolysis of pectin as followed viscometrically, but attention was directed chiefly to a striking differential effect of various solutes on rate of maceration and rate of toxic action.

With increasing concentration of solute, rates of both maceration and toxic action are reduced, but the latter is affected to a much greater degree. The results of two separate experiments with cucumber discs are shown in Table V. In both of these, acetone-precipitated preparations of *Bact. aroideae* enzyme were used. The range of concentrations of the solutes was such that the

effect on rate of maceration was negligible. The maceration time is indicated in the table by a vertical line. The progress of the toxic action was followed by the Neutral Red method.

The concentration below which the solutes had little effect in prolonging the life of the cells was that which just caused plasmolysis. For example, in a sub-plasmolytic concentration of KNO_3 (0·05 M.) there was little retardation of the killing effect, very few cells being alive after 20 minutes. In the presence

TABLE V

Effect of Concentration of Solute on Toxic Action of Bact. aroideae Enzyme

Solute and concentration.	Neutral Red Index at various periods of time (min.).						
	5	10	20	40	90	180	20 hrs.
Expt. 1. Potassium nitrate.							
0	0·5	0·1	0	—	—	—	—
0·05 M.	2–3	1	0·5	0·1	—	—	—
0·1 M.	5	5	5	5	—	—	4
0·15 M.	—	—	—	—	—	—	4–5
0·2 M.	—	—	—	—	—	—	4
Expt. 2. Glucose.							
0	—	2	0	—	—	—	—
0·2 M.	—	5	4–5	4–5	4	1–2	—
0·4 M.	—	5	5	5	5	2	—
0·5 M.	—	5	5	5	5	2–3	—

of 0·1 M. KNO_3, however, the majority of cells remained alive for more than 20 hours, this representing an increase of resistance to the toxic effect of the order of one-hundredfold. These protoplasts were only just rounded from the cell-wall.

The effect of plasmolysing solutions of glucose is equally well marked, though here the cells have begun to die in the plasmolysing solutions after 3 hours. This contrasts strongly, however, with the preparation without glucose, in which all the cells were dead within 20 minutes.

Results similar to those with potassium nitrate were given by sodium nitrate, sodium chloride, potassium acetate, and magnesium sulphate; the results with glucose were similar to those with sucrose and urea.

Parallel experiments with *B. cinerea* enzyme showed the same general behaviour, but with variations in detail. Sub-plasmolytic concentrations of the reagents (KNO_3, $MgSO_4$, glucose) were more effective in retarding the killing action of *Botrytis* enzyme than they were with bacterial enzyme and on longer treatment there was greater general toxicity than with the latter. The reactions with *Botrytis* enzyme were carried out at pH 5·6, which in itself was somewhat harmful, as shown by the gradual dying off of cells in the non-enzymic controls.

The effects detailed in this section are more readily interpreted as arising from an increased resistance of the protoplast than from an inhibitory effect of the plasmolysing agent upon the toxin. Only a small increase in concentration

of one of several agents is required to cause a very large resistance to killing. The resistance may, in fact, be a total immunity from lysis by the toxin under investigation, since the death of cells which have been plasmolysed for a long time may be due to factors other than this toxin.

D. Experiments with Extracted Protoplasts

By extraction of the protoplast from the plant cell it is possible to study the effect of the enzymic preparations directly on the protoplasmic membrane. Complications due to the cell-wall are thus removed.

Several workers have made use of the protoplasts isolated from plant tissues for experimental purposes, e.g. Levitt, Scarth, and Gibbs (1936); Törnävä (1939); Bennet-Clark and Bexon (1946). Tissues were plasmolysed, and when the protoplasts were sufficiently rounded off, they were liberated from the cell-walls by cutting or tearing the tissue apart.

The two tissues found to be most useful for this purpose were the mesocarp of cucumber and mesophyll of *Sedum spectabile*. From these large protoplasts were isolated in reasonable numbers by using plasmolysing concentrations of potassium nitrate. The technique with cucumber tissue was as follows. Cylinders of mesocarp were removed in a 0·8-cm. cork borer and the air spaces injected with tap-water under a pump. The tissue was plasmolysed for 60–90 minutes in 0·5 M. KNO_3, when the protoplasts rounded off evenly from the cell-walls. They were freed by sectioning the flaccid tissue and pushing the sections together with surrounding liquid into a watchglass of the plasmolysing agent. In some cases the tissue was pulled apart between mounted needles; in others sufficient protoplasts were released by the sectioning. Only a fraction of the bodies released were intact protoplasts. Many were tonoplasts, recognized by their hyaline appearance, perfectly spherical shape, and sharp outline; and many showed only fragments of cytoplasm on a hyaline sphere. Protoplasts, characterized by being uniformly covered with cytoplasm, were selected from the mixture under the low-power microscope and picked up by capillary pipette. Ten to thirty protoplasts were collected for each treatment. As a routine, three solutions were prepared for each experiment:

(*a*) enzyme preparation plus plasmolysing agent;
(*b*) heat-deactivated enzyme plus plasmolysing agent (first control);
(*c*) plasmolysing agent (second control).

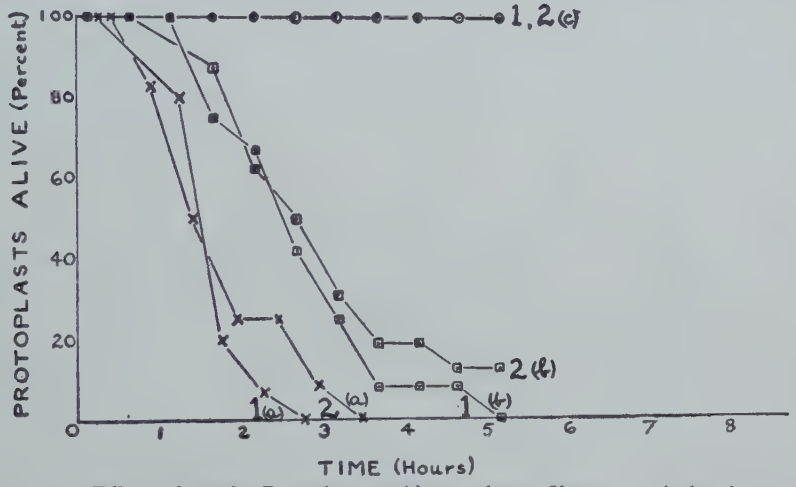
These were approximately isotonic with one another and with the plasmolysing solution from which the protoplasts were transferred. Although the agent used in the initial plasmolysis was potassium nitrate, the experimental solutions were usually made up with an organic substance, which does not retard macerating activity as do electrolytes such as potassium nitrate when used in concentrations required for vigorous plasmolysis. The above solutions were placed in three van Tieghem ring cells cemented on to a slide. Under

each cell, on the bottom of the slide, was drawn an indian ink grid, by which the position of the protoplasts was plotted on a reference grid on paper. The slide was held in a mechanical stage. Thus it was possible rapidly to locate each protoplast, and at intervals counts were made of those protoplasts still living, under low-power magnification.

Crude preparations of culture filtrates were used in these experiments.

(i) *Studies with Bact. aroideae enzyme*

Text-fig. 4 gives the results with extracted protoplasts of cucumber mesocarp. These had been plasmolysed in 0·5 M. potassium nitrate, from which they were transferred to solutions containing equal parts of 1·8 M. glycerol and (*a*) bacterial culture filtrate, (*b*) boiled culture filtrate, (*c*) original nutrient



Text-fig. 4. Effect of crude *Bacterium aroideae* culture filtrate on isolated cucumber protoplasts. (Two experiments, 1 and 2.) (*a*) Active preparation plus glycerol to 0·9 M., (*b*) Deactivated preparation plus glycerol to 0·9 M., (*c*) Diluted nutrient preparation plus glycerol to 0·9 M.

diluted to one-tenth. Diluted culture medium was used in (*c*) to ensure that the latter contained traces of salts present in (*a*) and (*b*) in case these should affect the behaviour of the protoplasts. The solutions had an osmotic concentration of approximately 0·9 M. glycerol, which is close to that of 0·5 M. potassium nitrate. The pH was 8·0.

At the beginning of the experiment all the protoplasts were spherical, and in preparation (*c*) they remained so throughout.[1] Later those in preparations

[1] Morphological changes were observed in protoplasts in all experimental solutions. Immediately after transference isolated protoplasts were spherical and cytoplasm was distributed regularly over the surface. After a short period of time various changes set in, involving displacements of cytoplasm about the transparent sphere which surrounds the vacuole and on which the cytoplasm is organized. Frequently the whole cytoplasmic layer slipped off much of the surface of the sphere and collected at one place on it. A full description of processes which involve cytoplasmic displacements following from plasmolysis of parenchyma cells inside a number of tissues is given by Germ (1932 and 1933).

(*a*) and (*b*) collapsed, and this failure of the semi-permeable membranes to maintain the spherical form was taken as the criterion of death. Protoplasts died approximately twice as rapidly in the active as in the deactivated enzyme solutions. Essentially the same result was obtained with extracted protoplasts of *Sedum spectabile*, isolated into either molar glycerol or molar urea. As before, the rate of killing in the unheated culture filtrates was about twice that in the boiled ones. The overall rate of killing was less than with cucumber protoplasts, and after 5 hours a few had collapsed in the (*c*) preparation.

(ii) *Studies with B. cinerea enzyme*

These experiments were carried out at pH 5·6–6·0, the region of optimal activity. A different supply of cucumbers were used, and whether because of

Text-fig. 5. Effect of crude *Botrytis cinerea* culture filtrate on isolated cucumber protoplasts. (Two experiments, 1 and 2.) (*a*) Active preparation plus potassium nitrate to 0·5 M. (*b*) Deactivated preparation plus potassium nitrate to 0·5 M. (*c*) 0·5 M. potassium nitrate.

a change in quality or because the tests were made at a more acid reaction is not clear, but these protoplasts collapsed more rapidly, even in the (*c*) preparations which contained no product derived from the fungus. The results of two experiments with protoplasts maintained in 0·5 molar potassium nitrate are shown in Text-fig. 5. In expt. 1 there was scarcely any difference in the death-rate of protoplasts in the active and deactivated enzyme solutions. In the second experiment the protoplasts in (*a*) died a little less than twice as quickly as those in (*b*).

In a further experiment with glycerol as plasmolysing agent, the results were similar to expt. 1 above. In two experiments with sucrose the protoplasts lasted longer, but the anomalous result was obtained that killing was more rapid in the (*c*) than in the (*b*) preparations.

The crude preparations used in these tests were of high activity, macerating

fresh cucumber discs in about 6 minutes and causing the death of all but occasional protoplasts *in situ* in 15 minutes. When such discs were plasmolysed in 0·9 M. glycerol or urea or sucrose, the rate of maceration was not sensibly diminished; in 0·5 M. KNO_3 it was retarded to 35 minutes. The above experiments show the same general trend as did those with *Bact. aroideae*, but less reliance can be placed upon them in view of the unsatisfactory behaviour of the control (*c*) preparations.

The overall suggestion is that a thermostable substance or substances toxic to protoplasts were present in the culture filtrates used. In addition there is an indication that a thermolabile toxin was also present. Since, however, the normal susceptibility of parenchyma cells to the rapid killing was completely altered by plasmolysis, it is difficult to say whether this toxin is the toxic factor associated with enzyme activity on unplasmolysed cells. It was the resistant behaviour of extracted protoplasts which led to the experiments with plasmolysed tissues already described in section C (iii). Protoplasts plasmolysed *in situ* were found even more resistant to the toxic action than isolated protoplasts (cf. Table V).

E. Discussion

The experiments described in section C clearly show a parallelism between activity as measured by (i) reduction in viscosity in pectin solutions, (ii) maceration of higher plant tissues, and (iii) killing of normal (unplasmolysed) cells, by enzymic preparations from both *Botrytis cinerea* and *Bacterium aroideae* under a range of experimental treatments. There is little doubt that a polygalacturonase is responsible for viscosity reduction by *Botrytis* solutions, since Jermyn and Tomkins (1950) found that a *Botrytis* enzyme degraded both pectin and pectate eventually to galacturonic acid by hydrolysing 1:4 glycosidic bonds and that viscosity of the solutions was reduced to that of water when some 10 per cent. of such bonds were hydrolysed. The evidence of Wood (1955) indicates that the bacterial enzyme is a depolymerase, which though reducing the viscosity of pectin liberates no galacturonic acid and few reducing groups even after prolonged action. It is possible that a depolymerase is also present in *Botrytis* preparations.

The parallelisms summarized above strongly indicate that these pectin-degrading enzymes also macerate tissue, i.e. that they are protopectinases. Quantitative agreement of differential activities as measured by the two tests was not always close, as has been pointed out with reference to the influence of pH in section C, p. 357. These differences seem to be largely resolved, however, if the non-linear relationship between macerating activity and concentration is taken into account. This relation, where dilution of enzyme leads to less loss of activity than would be expected on the basis of the linear law, is probably due to heterogeneous distribution of the substrate in tissue limiting the rate of diffusion of enzyme, and possibly also to adsorption of enzyme. Another factor making real comparison uncertain is, of course, non-identity

of the substrates. Protopectin is probably of higher molecular weight and is unlikely to have a degree of methylation equal to that of a test pectin solution.

The above parallelisms also give strong indication that the pectin-degrading enzymes are likewise directly responsible for killing the cells. The close correspondence between killing and maceration (see especially Text-fig. 1 and Pl. X, Figs. 1–2), originally established by Brown (1915) for extracts of *Botrytis* germ-tubes, was confirmed and extended to *Bacterium aroideae*. It is noteworthy that the bacterial enzyme preparations were not toxic to an alga (Cladophora) and a liverwort (Fegatella) tested, and these tissues were likewise not macerated. Brown (*loc. cit.*) reported on the resistance of certain algal and Bryophyte tissues to *Botrytis* enzyme.

The experiments described in section D, in which protoplasts were isolated from tissues, were intended in conjunction with enzyme deactivation tests to prove directly the presence or absence of a colloidal toxin. The plasmolysis necessarily involved in this technique, however, caused a very great resistance to the killing effect and so completely altered the normal maceration-killing relationship. Owing to this factor it is difficult to say whether the toxicity which generally increased the rate of death of protoplasts in active enzyme solutions as compared with those in deactivated solutions is really the same as the toxic factor which affects unplasmolysed protoplasts. Crude culture filtrates were used in these tests and a toxicity due to thermostable substances was shown.

The effect of plasmolysis in greatly retarding the killing action, in relation to the macerating action, is very striking. It is, in fact, the first evidence which has been obtained of a differential effect on maceration and killing. One interpretation of the protective action of plasmolysis would be as follows. If the walls of unplasmolysed cells were sufficiently macerated as to afford no mechanical support to the protoplasts, the latter might merely burst. In that case there would be no need to postulate a toxin at all. However, microscopic examination does not support this view. Pl. XII, Figs. 1–3, shows the effect of the action of a bacterial enzyme preparation on the same field of (unplasmolysed) cells of *Sedum spectabile* mesophyll at three consecutive intervals of time. The tissue was pre-stained in neutral red, and the toxic action is demonstrated by progressive disappearance of the stain from the cells. After 1 hour's treatment, when all the cells were dead, the wall outlines remain unaltered. In *Sedum* tissue, even after prolonged enzyme treatment, the vacuole membrane (tonoplast) of occasional cells remained semi-permeable. Pl. XII, Fig. 4, shows such a cell, after treatment in neutral red in 0·5 M. KNO_3 solution, following extensive enzyme action. The vacuole has plasmolysed and stained up inside a disorganized mass of chloroplasts and other protoplasm. This seems to indicate that the vacuole membrane itself is not susceptible of enzyme action, but is probably usually broken up following death of the cell.

The differential effect on maceration and toxic action was produced at plasmolysing concentrations by a number of salts and organic substances

(section C (iii)). The toxin was strongly retarded at and above plasmolysing concentrations, though it was affected negligibly, as was the macerating enzyme, at sub-plasmolysing concentrations. It is very unlikely that the toxin should become especially sensitive to a variety of substances at these concentrations, but rather that at about the stage where plasmolysis is induced the protoplast develops a much increased resistance to penetration of the toxin.

If then the pectin-degrading enzymes are responsible for killing the cells, the substrate on which they act must be assumed to be of pectic nature. The pectic material must either form part of the ectoplast membrane, or the membrane must be permeable to the enzyme, giving access to internally situated molecules. The plasmolysis experiments could be interpreted as indicating that the outermost molecular layer is not pectic, but that the membrane becomes impermeable on plasmolysis to penetration of the enzyme. Pectic material may be associated with the ectoplast, such that if dissolved out the semi-permeable properties of the membrane are lost, or it may be present as a major constituent of parenchyma protoplasts, leading, on hydrolysis, to lysis of the protoplast.

It is to be noted that no mention of pectin as a structural component of plant protoplasts, or indeed of its presence therein, is made in modern treatments of protoplast structure (Scarth, 1942; Frey-Wyssling, 1948). An interesting case which appears to be a parallel phenomenon in animal tissues is that of the alpha toxin of *Clostridium welchii*, which causes necrosis of animal tissue and is very toxic. The effect is apparently explicable on the basis of lecithinase activity, and a parallelism exists between increased toxicity and increased rate of hydrolysis of lecithin on purification. Lecithin is an essential component of animal cell-walls (Gale, 1951).

An attempt was made to find a substance capable of macerating tissue without killing the cells. A number of reagents were tried which react with calcium, in order to bring about solution of the middle lamella, viz. ammonium oxalate and sodium pyrophosphate, fluoride, carbonate, and hexametaphosphate. Some of these are used for extracting cell-wall pectins at elevated temperatures (see Baker and Woodmansee, 1944, for hexametaphosphate and other phosphates). The reagents acted very slowly at room temperatures; maceration was effected in cucumber discs overnight with hexametaphosphate, pyrophosphate, and oxalate, but the cells were also killed. Potato discs were unaffected overnight in any of these reagents, whilst turnip discs just lost coherence in oxalate and hexametaphosphate, a few cells being alive in the latter and none in the oxalate. In turnip tissue this persistence of living cells for some time after loss of coherence also occurs after treatment with soft-rotting enzymes. Thus no positive results were obtained by this approach.

The hypothesis is put forward that the death of higher plant cells accompanying maceration by *Botrytis cinerea* and *Bacterium aroideae* enzymes is explicable by direct hydrolysis of pectic material present in them and accessible in unplasmolysed living cells. Further testing of this hypothesis would

be possible by the use of highly purified polygalacturonase and pectin-depolymerase enzymes.

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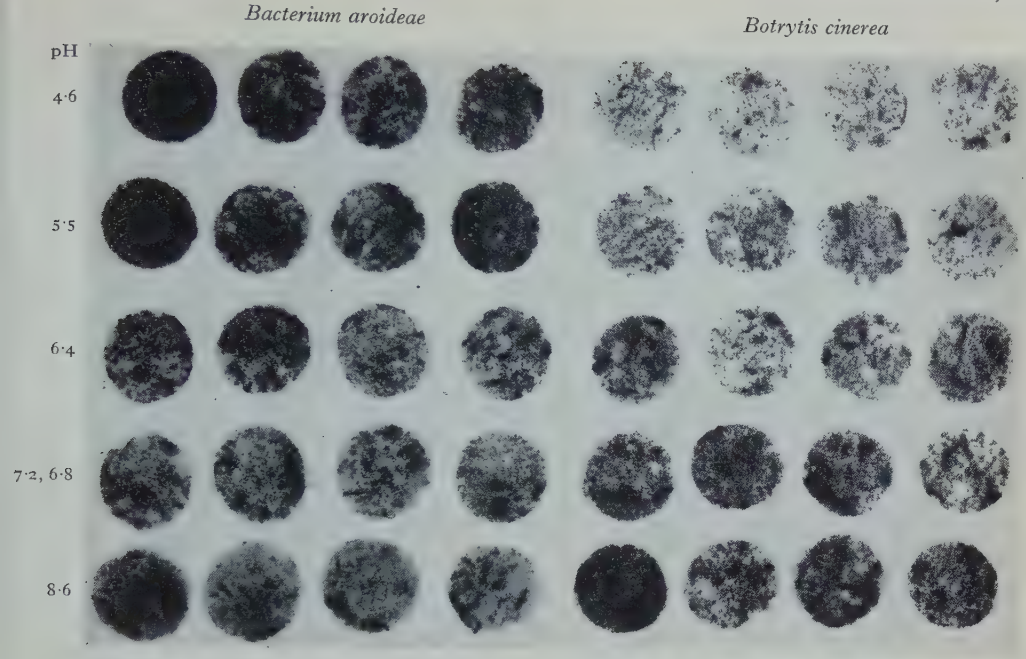

FIG. 1. 25 mins. treatment

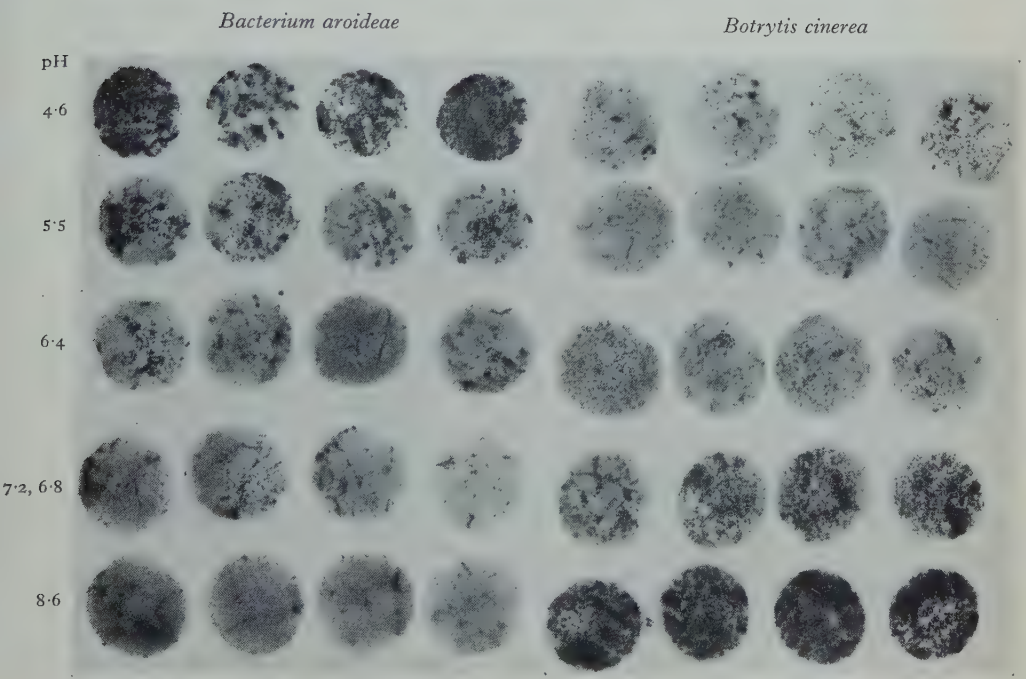

FIG. 2. 90 mins. treatment

Botrytis cinerea and *Bacterium aroideae* Enzymes

Relation of pH with Toxic Activity, as followed by the Neutral Red Technique on Discs of Potato Tissue

H. T. TRIBE

Further Studies on Heterophylly in *Callitriche intermedia*: Leaf Development and Experimental Induction of Ovate Leaves

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Plate XIII and two Figures in the Text

ABSTRACT

The forms of successive primordia in the development of linear and ovate leaves are described and discussed in relation to primordia in crowns where a transition is occurring between ovate leaves and linear leaves. Ovate leaves are induced on submerged axes, originally bearing linear leaves, by submerging these axes in 30 per cent. sea water. The results are discussed in relation to possible interpretations of heterophylly in *C. intermedia*.

INTRODUCTION

A CLEAR picture of the development of a leaf is given for tobacco by Avery (1933). At a length of about 1 mm. the primordium is linear in form and consists chiefly of midrib, the lamina developing from this point through marginal activity. By the time that the primordium approaches a length of 5 mm., the number of cell layers in the adult lamina is already established. The sinuate outline of the lateral walls of the epidermal cells does not develop until long after cell division has ceased. By marking the young leaf and observing the pattern assumed during development, Avery concluded that the final shape of the leaf depended upon (1) localized growth, that is a differential distribution of growth in its various parts; (2) polarized growth, that is greater growth in one dimension than in another in various parts of the leaf. From these conclusions it is evident that the leaf primordium is provided with a comprehensive mechanism which permits of potential development into a variety of shapes. Ashby (1948) has pointed out that the

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shape of the mature leaf is determined by three criteria: (1) the shape of the primordium; (2) the number, distribution, and orientation of the cell divisions; and (3) the amount and distribution of cell enlargement.

Where a plant exhibits heterophylly it is of interest to know at what stage in development the change in form occurs. Cross (1937) in a study of the cataphylls, transitional forms, and foliage leaves of *Viburnum rufidulum* found that the primordia of all types were identical up to the 500 μ stage. McCallum (1902) states that the primordium of *Proserpinaca palustris* can change into either a water leaf or an air leaf at a length of from 3 to 4 mm. Burns (1904) showed that the 'plastic' primordium has a midrib with lateral lobes. If a water leaf is to develop, these lobes grow out into long filaments, while the air leaf is formed by the intercalation of lamina between the midrib and the lobes, the latter eventually forming the serrations on the margin of the lanceolate leaf. Hegelmaier (1864), referring to leaf development in species of *Callitriche* which showed heterophylly, states 'at first the young leaves assume an oval shape in all types'. He suggests that the differences in leaf form are concerned with the differential expansion of cells, especially in relation to a different development of the air spaces in the linear and ovate leaves. Ashby and Wangermann (1950) state that in *Ipomoea* the factors which cause a difference in cell size between upper and lower leaves operate (*a*) early in the phase of cell division before the leaf reaches an area of 0·04 sq. cm., and also (*b*) at the point where cell expansion begins, by prolonging (in an upper leaf) the phase of cell division and delaying the onset of cell expansion.

While these facts seem to indicate that the very early development of the primordium is irrelevant to the problem of heterophylly, the work of Abbé, Randolph, and Einset (1941) shows that, in the case of maize, the shape of the shoot apex is important. These workers showed that the leaf blades, numbered 6–12 (in the order of their formation), were progressively wider and that the increase in width was correlated with a progressive increase in width of the shoot apex. The increase in size of the shoot apex was related to increase in cell number, while cell size and nuclear size remained constant. Again, a familiar feature of the change from the vegetative to the floral condition in many plants is the striking change in the form of the apex.

Hammond (1941) studied the change in form of leaves of a number of species (including a species of *Sagittaria*). She applied Huxley's formula for relative growth according to the equation

$$y = bx^k,$$

where y = length of leaf,
x = breadth of leaf,
b is a constant expressing the initial relationship between x and y,
k is a constant relationship between the growth rates of x and y.

Out of 12 species, in only 1 case (*Vallisneria*) was a linear relationship not obtained when the logarithms of length and breadth were plotted. In most cases of leaf development it would appear that the relationship of length and

breadth follows the formula quoted above, the slope of the line representing k. It is clear that when the slope is greater than 45° the leaf is lengthening and that when the slope of the line is below 45° the leaf is becoming broader. Hammond's results show clearly how leaves of widely different form may arise from primordia identical in shape. In Hammond's work, plots from a series of leaves of different ages fell along the same line as plots from developmental measurements of single leaves. Richards (1934) discussess the validity of the assumption that a series of succeeding leaves at any one time represents the course of one leaf in its development. He says that only in exceptional cases is this so. In the case of *Callitriche intermedia*, however, where the shape of the fully developed leaf is remarkably uniform provided the conditions of submergence or emergence are maintained, it is a fair assumption that a series of leaves at any one time does represent the stages in development of a single leaf. Again the value of such a study in the case of heterophylly is that when successive leaf dimensions are plotted as discussed above, then a change in direction of the plots enables the first leaf which shows a fundamental change in form to be located with reasonable accuracy.

In the present work information is sought concerning the rate of initiation of leaf primordia. Allsopp (1953) studied the rate of leaf formation of *Marsilea* sporelings grown on nutrient medium containing various sugars. He found that the rates of leaf formation followed approximately the same order as the growth rates of the plants. Arney (1953) shows how the number of primordia in the apical bud of a variety of *Fragaria vesca* can vary seasonally due to a difference in the rates of initiation of leaf primordia and emergence from the bud. It appears that one primordium is formed between the beginning of November and the end of January. Arney also shows that the rate of increase of the primordium is exponential at first and then the primordium enters a phase of high but uniform rate of elongation.

In a previous paper (Jones, 1955) some aspects of variation in mature leaves of *C. intermedia* were studied as well as features connected with submergence of shoots bearing ovate leaves at the crown. In the present work ovate-crowned shoots were submerged under conditions of light and temperature which were known to hasten the formation of linear leaves. Samples of crowns were taken at short intervals and the form and length of developing leaves studied by means of dissections and longitudinal sections. Studies were made, for purposes of comparison, with leaves from linear crowns and ovate crowns. Finally, the rate of initiation of leaf primordia in submerged, ovate-crowned shoots was compared with that in floating crowns.

Leaf Development

(*a*) *Leaf shape during the development of linear and ovate crowns and during the transition from ovate to linear leaves*

Methods. The most rapid production of linear leaves in the submerged ovate crown in the laboratory (4 days) had been at 25° C. and under conditions of summer daylight supplemented by artificial light at night (Jones,

1955). It was decided to adopt conditions as similar to this as possible in order to obtain crowns in transitional stages. As this work was to be carried out in January, the daylight was augmented by two 60-watt gas-filled lamps each at 6 in. from the shoots. This lamp distance was also maintained at night.

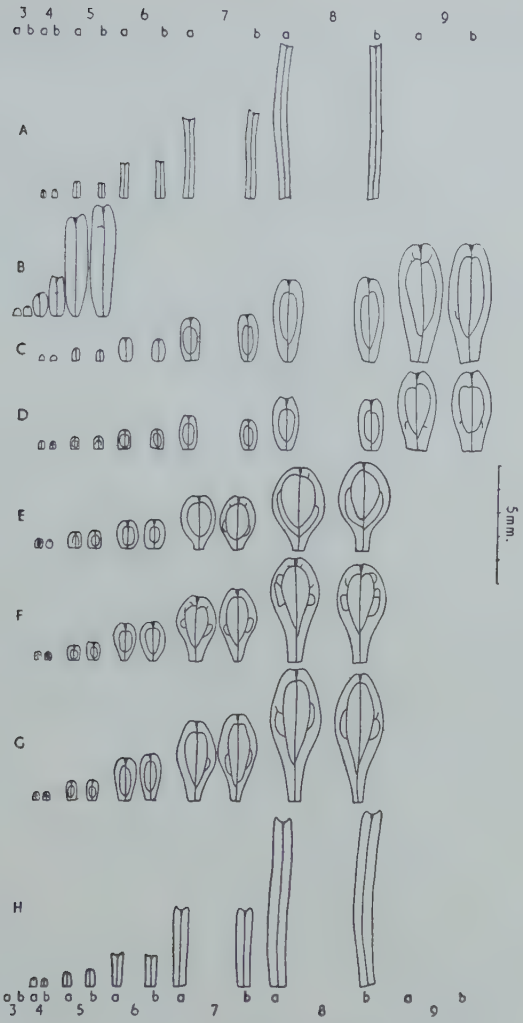
Ovate-crowned material of *Callitriche intermedia* was collected from an ox-bow of the lower Rheidol on January 5, 1953. Shoots (each with 5 internodes of length 1 mm. or greater) were tied together loosely with cotton thread in bundles of 7. Ten of these bundles were made up and each was attached to a glass rod and submerged in tapwater in a 5½-litre tank. The water in the tank was maintained at a temperature of 25° C. using a water heater and thermostat as described previously (Jones, 1955), the water being aerated for 15 minutes morning and evening.

The experiment was set up at 12.30 p.m. on January 5. At each of the times specified in Appendix Table I a bundle of 7 shoots was removed from the tank. The crowns were then fixed immediately. This material and of normal ovate and linear crowns for comparison was fixed in formalin-acetic-alcohol. After 24 hours leaves were decolourized sufficiently to observe details of venation. For dissection, the crowns were taken through changes of 50 and 20 per cent. alcohol to water in which they were dissected under a binocular microscope using needles sharpened to triangular points (Sharman, 1947). The pairs of leaves were removed in order of decreasing age until 3 pairs of primordia remained at the apex of the shoot (it was not found practicable to remove further primordia). The dissected leaves were then mounted in water under a cover-glass and drawn with the aid of a camera lucida. Material for sectioning was embedded in paraffin wax and longitudinal sections cut at 10 μ. These sections, which were cut in a plane through alternate leaf pairs, were then stained in Heidenhain's haematoxylin after mordanting with iron alum.

Results. Leaf dissections are shown in Text-fig. 1 (A, B, C, D), while Pl. XIII shows photomicrographs of longitudinal sections of ovate and linear crowns (A 1, 5) and also some of the transitional crowns (A 2, 3, 4). The approximate number of internodes each longer than 1 mm. in the experimental material on successive days is shown in Appendix Table I. The last sample showed linear leaves at the crown in all cases, while in 2 crowns leaves of the inner crown were 1-veined. Appendix Table II shows comparisons of lengths of the 7th or 8th primordium initially and after 43 hours.

(*b*) *Rates of leaf initiation and axis elongation in floating and submerged ovate crowns, together with some further observations on the development of leaf shape*

From the previous work there arose a possibility that the transformation from ovate to linear leaves was accompanied by a slowing down or temporary cessation in the rate of initiation of leaf primordia. The following experiment was carried out to investigate this point as well as to provide additional data on two further features. The first was the elongation of the axis on submergence



TEXT-FIG. 1. Leaves dissected from the crowns of *C. intermedia* shoots

A–D. January.
A. Linear crown.
B. 6½ days after submergence of an ovate crown (Leaf Development Experiment a.)—A4 of Plate XIII.
C. 2½ days after submergence of an ovate crown (Leaf Development Experiment a.)—A3 of Plate XIII.
D. Ovate crown.
E–H. August.
E. Ovate crown.
F. Ovate crown (Floating in Leaf Development Expt. b.).
G. 8 days after submergence of ovate crown (Leaf Development Experiment b.).
H. Linear crown.

Numbers refer to leaf primordia in the order of their appearance (in A for example, 3 pairs of primordia remained on the apex after dissection.

compared with that of a shoot whose crown is at the surface. Secondly, more information was required than was previously available on the form of the transitional leaves under conditions that approximated more closely to the natural than was the case in the last experiment.

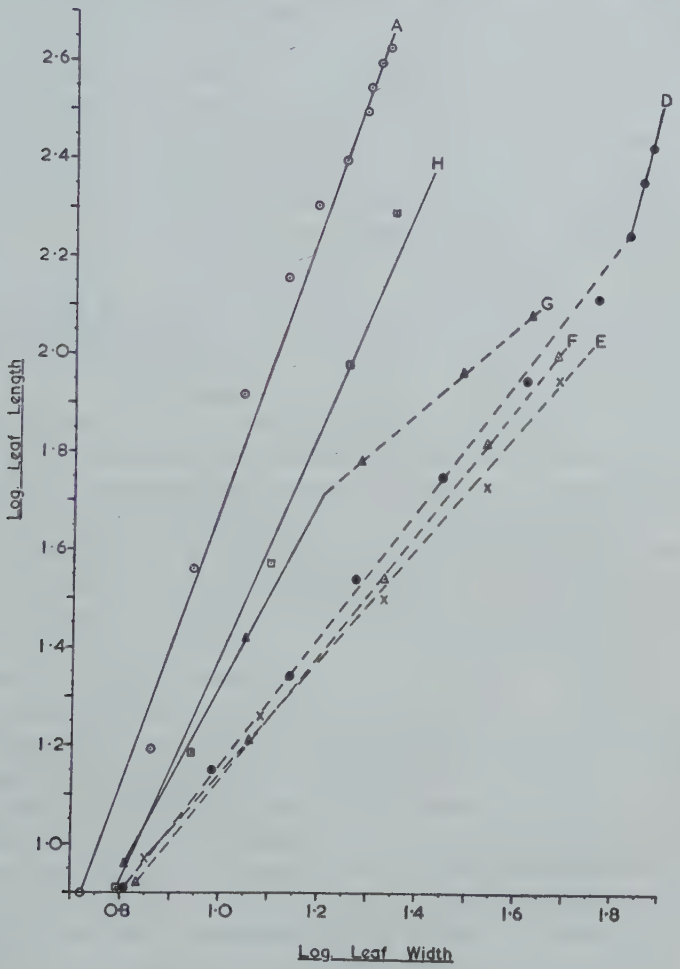
Methods. Ovate-crowned material was obtained from an ox-bow of the lower Rheidol on August 12, 1953. Eleven crowns were fixed immediately in formalin-acetic-alcohol. Twenty-two similar shoots, each with at least 10 internodes, each exceeding 1 mm., were selected. The first internode shorter than 1 mm. (that is the internode next above that which measured 1 mm. or more) was marked by means of a short length of cotton thread tied loosely around it. Eleven of the shoots were tied in such a way, to a glass rod placed in a $5\frac{1}{2}$-litre tank of tapwater, that the crowns were floating at the surface. The other 11 shoots were tied to a second glass rod so that the crowns were some 10 cm. below the surface. The tank was arranged out of doors with a glass plate placed some distance above the top of the tank to provide shelter from rain but not to interfere with access of air to the surface water. The water was not otherwise aerated, but on days with bright sunshine the tank was shaded with thin paper. In order to compensate for growth of the floating axes, water was added to the tank at intervals. Without this precaution growth of the axes would have resulted in crowns being pushed sideways and possibly submerged (due to the bases of the shoots being fixed to the glass rod). To prevent the crowns of the submerged shoots from reaching the surface these shoots were tied lower to the supporting glass rod during the course of the experiment. On August 20 all shoots were fixed in formalin-acetic-alcohol. On August 18 11 linear crowns from an ox-bow of the lower Rheidol were also fixed. Unfortunately, on later examination several of these latter were found to possess 3-veined leaves in the crown, due it is believed to these crowns having reached the surface and then been submerged in a subsequent rise of water in the ox-bow. There being no other material available, these crowns were used for comparison.

Results. Appendix Table III gives the corresponding numbers of nodes in submerged and floating shoots at the end of the experiment. Lengths of shoots are given in such a form as to show where the elongation of the axis has occurred, that is with respect to above or below the base of the original crown. Appendix Table III also gives, for comparison, the numbers of nodes in ovate and linear crowns at August 12 and August 18 respectively and ovate and linear crowns at January 14. Text-fig. 1 (E–H) shows dissections of samples of the experimental floating and submerged crowns compared with naturally occurring ovate and linear crowns. Text-fig. 2 shows the graphs obtained when logarithms of average leaf length and breadth are plotted, the youngest primordium which was measured being the 4th.

(*c*) *Discussion*

From Text-fig. 1 it is seen that the 4th primordia of linear and ovate crowns are similar in form. In the 4th or 5th primordium begins the vein distinction.

Where a leaf will become ovate, the 3 veins are discernible. In successive leaves older than the 4th, the leaf shape remains fundamentally unaltered, apart from the relatively narrow leaf base in older leaves. The maintenance of the leaf shape is shown also by the plots of the logarithms of length and



TEXT-FIG. 2. Plots of logarithms of leaf dimensions. (Starting with 4th leaf.)

Leaves taken from ovate, linear and ovate/linear transitional crowns. (For explanation see Text-Fig. 1.)

Solid and interrupted lines represent phases of linear and ovate leaf development, respectively.

breadth (Text-fig. 2), the slope of the line being of the order of 45°. In the development of linear leaves, after the 4th primordium, the leaf becomes much more elongated. This is shown in the steep slope of the plots of logarithms of leaf length and leaf breadth. In both cases the straight-line nature of the plots shows that the leaf dimensions are altering in a uniform manner. In

the older primordia of the ovate crown (D), the sharp upward tilt of the plot indicates the essentially linear growth type of these leaves, indicating the recent arrival at the surface of the crowns.

From an examination of the longitudinal sections of transitional crowns from Expt. (*a*), the first noticeable change is to be seen after 43 hours (Pl. XIII, A2). The 7th or 8th primordium has become noticeably longer. The angle which the leaf makes with the axis is more acute in A2 than in A1. After $2\frac{1}{2}$ days the 8th leaf pair has become considerably elongated. The crown A3 now bears a close resemblance to the linear crown (A5). After $6\frac{1}{2}$ days the distinction in length between successive primordia is even more marked (A4). All the crowns by this time bear linear leaves, while in 2 crowns 1-veined leaves are present (Text-fig. 1B).

In Pl. XIII the appearance of the section A4 when compared with A1 suggests that no new leaf primordia have been initiated since the start of the experiment and that the existing primordia have attained an abnormal length. The development of the flower primordia to a large size close to the apex also suggests that leaf-primordium initiation has ceased.

In Expt. (*b*), which was designed to provide more information on this point, it may be seen (Appendix Table III) that the total number of internodes in floating and submerged crowns remains constant, under the conditions of the experiment (14·9 as against 15·4). The difference between the shoots when submerged and floating lies in the distribution of these internodes. In the submerged shoot the crown nodes have decreased in number in compensation for the greater number of nodes below the crown as a result of greater growth of the submerged shoot. In the shoot where the crown is floating, the number of nodes is higher and agrees closely with the number in the crown of the control sample (E). The smaller amount of axis growth in the shoot with floating crown has accounted for the small number of nodes formed below the crown. The question arises as to whether the linear crown eventually formed on submergence will contain a smaller number of nodes than the ovate crown. The sample of linear crowns taken on August 18 is not a reliable comparison owing to the presence of some 3-veined leaves, but it may be significant that this sample does contain an average of only 10 nodes. The winter samples of ovate and linear crowns show virtually the same node number (13·7 and 14·1), so that any distinction in the node numbers of the crown occurs other than during the winter. In winter the number of ovate crown nodes is slightly greater than that in the summer. By the end of the experiment about 3 leaves have been initiated in both the submerged and floating shoots (the control crown contains 12 nodes and the submerged and floating shoots shown some 15 nodes each above the cotton loop marking the base of the original crown).

The shoot measurements show that since the beginning of the experiment growth below the original crown has been equal in both floating and submerged shoots. The very much greater growth of the submerged shoots has resulted from a higher rate of expansion of those internodes which were unexpanded

at the start of the experiment. Not only have more internodes expanded in the submerged shoots but the expansion of each of the internodes is greater than in the internodes of the floating shoots.

The plots of logarithms of leaf length and breadth give a straight line at an angle of about 50° with the horizontal for the floating shoot. For the submerged shoot, however, the plots form two straight lines at different angles. This latter shows that the 4th, 5th, and possibly 6th primordia are developing as typically linear leaves while the older primordia are still showing a slope indicative of ovate development. Since it is likely that the 4th primordium can develop into either an ovate or a linear leaf, then with 3 primordia formed since the beginning of the experiment, it might be expected that the 7th primordium would be developing into a completely linear leaf. The fact that only the 5th or 6th primordium is linear suggests that there is a time-lag between the moment of submergence and the operating of the mechanism which alters the shape of the leaves. That there is a lag is also suggested from the photographs of submerged ovate-crowned shoots, where it may be seen that the leaf movements typical of the submerged crowns do not start until the day following submergence (Jones, 1955). In none of the submerged crowns in the experimental material are single-veined leaves observed. It would appear that separate mechanisms control the leaf shape and the venation.

The Experimental Induction of Ovate Leaves in Submerged Crowns of *Callitriche intermedia*

Heterophylly in *Callitriche intermedia* has up to this point been considered mainly in connexion with the transition from ovate to linear leaves, this being far more easily arranged experimentally than the reverse change. The latter only occurs naturally when the linear crown has risen to the surface of the water. If, however, the ovate leaves can be induced to form while the crown is still submerged, then the means by which this is achieved should throw some light on the mechanism involved in the natural change.

The formation of ovate leaves on submerged linear crowns has been achieved in the present work using a modification of McCallum's methods. McCallum (1902) was able to induce the aerial type of leaf in *Proserpinaca palustris* by submerging shoots bearing 'water' leaves in nutrient solutions of a concentration not quite sufficient to plasmolyse the tissues. In a recent paper Allsopp (1953) has shown that the period of heteroblastic development in sporelings of *Marsilea* may be shortened by increasing the concentration of sugar in the nutrient medium. Allsopp maintains that the operative factor is nutritional and not osmotic (a shortening of the axis with higher sugar concentration is, however, attributed to higher osmotic concentration). At the same time this worker points out that at high sugar concentrations the sporelings, although growing in a liquid medium, possessed the features of the land form while in low sugar concentrations the features were those of the water form.

Methods. In the present work, instead of a nutrient solution, diluted sea

water was used as a medium for the immersion of the linear-crowned shoots. This provided a medium of suitable osmotic pressure as well as a convenient, balanced solution. By immersing leaves from linear crowns in sea water of a range of dilutions, it was established that 35 per cent. sea water caused plasmolysis of the tissues while no plasmolysis occurred in 30 per cent. sea water. (Incipient plasmolysis occurred in 0·29 volume molar sucrose solution, indicating that the osmotic pressure of the leaf tissues was 7·8 atmospheres (at 20° C.).) Leaves of ovate crowns were examined in the same way, but determinations of the osmotic pressure could not be made with the same accuracy, as the ovate leaves were thicker and more opaque. It appeared, however, that the osmotic pressure of ovate leaf tissue was approximately the same as that of the linear leaves.

Ovate and linear-crowned shoots were immersed in 30 per cent. sea water while controls were set up in a similar manner with tapwater substituted for the sea water. Shoots were attached to a glass rod placed in a 6 × 1 in. test-tube, there being one shoot to a tube. Test-tubes were then placed at random in a rack near a window. Two series were set up, the second with the object of obtaining material for sectioning and for stomate counts.

Series 1 (*July 9–July 30, 1951*). Ovate-crowned material was obtained in this case from the main stream of the Rheidol, low water having prevailed for some time. Linear-crowned material from submerged shoots was obtained from the same locality.

The results of these series are given in Appendix Table IV. On July 26 (after 17 days) ovate leaves were recorded on one of the hitherto linear crowns. By accounting for the evaporation from the test-tubes, the concentration of sea water was estimated to be approximately 37 per cent.

Series 2 (*April 8–May 12, 1952*). Linear-crowned material was gathered from the Rheidol main stream and ovate-crowned shoots from an ox-bow of the lower Rheidol. A check on the evaporation from the test-tubes was made by weighing certain of the tubes at intervals. In this way the concentration of the diluted sea water could be estimated at any given time (Appendix Table V). Appendix Table VI shows the details of the experiment. The variation in the volumes of liquid in the test-tubes results from the decision to adopt a uniform height of liquid throughout despite inequalities in test-tube size. Evaporation from the sample tubes containing sea water was assumed to be representative of the other tubes of sea water. At the end of the experiment crowns which were required for further examination were fixed in formalin-acetic-alcohol. From the induced ovate crowns photographs were made of the series in leaf form from linear to induced ovate with normal linear and ovate leaves for comparison (Pl. XIII, C 1–6). From this series an investigation was made into stomate and epidermal cell frequencies (Appendix Table VII) and form of epidermal cells (Pl. XIII, D 1–4). Stomatal indices from normal linear and ovate fully developed leaves are also included for comparison. Some of the induced ovate crowns were sectioned for comparision with a normal (linear) crown (Pl. XIII, B 1, 2).

The results of Series 2 in Appendix Table V show that ovate leaves were recorded on linear crowns submerged in the sea water after 11 days. At this time the concentration of the diluted sea water was between 32 and 33 per cent. By May 12, 10 ovate crowns had been produced by the action of the sea water. The remaining 5 shoots were dead. By May 12 the originally linear crowns in tapwater were still linear and the originally ovate crowns in tapwater were linear (2) and ovate-linear (1). Of the originally ovate crowns in sea water, 2 were dead but all had ovate crowns.

From Pl. XIII, C, it can be seen that the formation of the ovate leaves is a gradual process,the early transitional leaves having a slight enlargement of the base, this becoming more pronounced in succeeding leaves until leaf C 3 may be described as ovate with a linear tip. Leaf C 4 is ovate. The induced ovate leaves recorded in these experiments have never exceeded 3 mm. in length and are 1-veined. The stomate counts recorded in Appendix Table VII show that the assumption of the ovate form results in a general increase in the stomatal index, although the index for C 4 is less than that for C 3. The stomatal index of the fully developed linear leaf is very low compared with that of the normal fully developed ovate leaf.

In surface view the normal ovate leaf has isodiametric epidermal cells (Pl. XIII, D 3), while the normal linear leaf epidermal cells are markedly elongated in the direction of the length of the leaf (Pl. XIII, D 4). The epidermal cells of the induced ovate leaf C 4 are isodiametric. (The anticlinal walls of epidermal cells of normal ovate leaves are sinuate. This feature occurs in some of the epidermal cells of induced ovate leaves and also occasionally in normal linear leaves.)

Longitudinal sections of the induced ovate apex (Pl. XIII, B 1) show that the cells are in a vegetative condition, contrasting strongly with the section of B 2, a linear crown.

Discussion

It is important to establish the degree of similarity between normal ovate leaves and the ovate leaves induced in these experiments. The forms of the two types of leaves are similar. Further points of similarity are the high stomate frequencies of the induced leaves and the isodiametric epidermal cells. The stomatal index of the induced ovate leaves approximates to that of C 5 (Pl. XIII) an immature normal ovate leaf of about the same size. The epidermal cell frequencies are also of the same order. This would suggest that the induced ovate leaves have been arrested at an early stage in development. A curious feature is that C 5 has a lower stomatal index than the fully developed normal ovate leaf. This probably arises because the lower half of the leaf is not yet fully developed (shown in the higher cell frequency) and many of the future stomates have not yet been differentiated. C 6, an immature linear leaf of the same age (8th node) as C 5, is relatively more mature as seen by the greater uniformity in cell frequencies from tip to base of leaf and the

closer agreement of cell frequency with the mature leaf than shown by C 5.

The form of the transitional leaf C 2 is now partially explained. A feature of this is that the tip resembles that of a linear leaf while the base is that of an ovate leaf in appearance and in stomatal index. The greatest alteration in the form and structure of the leaf is, of course, situated in the relatively less developed basal part of the leaf. The lower epidermal cell frequency of C 4 compared with C 3, together with the smaller leaf size, suggests that cell division stopped at an earlier stage in C 4. This is probably accompanied by a lower level of differentiation of tissues, this suggestion being supported by the appearance of the leaves in the longitudinal section (Pl. XIII, B 1). The lower stomate frequency of C 4 compared with C 3 is probably related to the same feature.

In no case did an induced ovate leaf become 3-veined. A curious feature is that an ovate-crowned shoot immersed in diluted sea water formed axillary buds, the leaves of which were 1-veined. While the action of the sea water probably prevents differentiation in the very young primordia, as discussed above, it seems unlikely that this would give rise to a 1-veined condition instead of 3-veined. It is again suggested that the form of the leaf and the venation are characters which are developed independently.

The apex of the induced ovate shoot is not active meristematically. This non-functioning apex is also associated typically with the development of axillary buds. In this case the treatment with sea water has had the same effect (as regards development of axillary buds) as removal of the apex. The ovate-crowned shoot which was immersed in sea water also showed marked development of axillary buds.

McCallum's explanation of the action of high osmotic pressures in this connexion was that the withdrawal of water osmotically produced a similar condition to that created by the withdrawal of water by transpiration on exposure of the crown to the air. The logical corollary would be to explain the change in leaf form in terms of turgor changes at the leaf primordium, with turgor changes directly influencing the shape of the leaf. No evidence for this view can be put forward in the present work, but it is an approach worthy of further study, as would be a study of the osmotic pressures of the tissues of floating and submerged leaves.

A further suggestion offered is that the turgor changes affect auxin production and translocation, these in turn affecting the leaf form (under natural conditions the turgor changes would occur in the older leaves when these break water surface and are exposed to the air). That an auxin mechanism is involved in the induction of ovate leaves by diluted sea water is suggested by the correlation of the axillary bud development with the non-meristematic induced ovate apex. Furthermore, it would seem from studying the leaf movements and growth of submerged ovate-crowned shoots (Jones, 1955) that auxin fluctuations do indeed play an important part in the reverse change from ovate to linear leaves.

Summary

1. The leaf primordia of ovate-crowned and linear-crowned shoots of *Callitriche intermedia* are identical up to approximately the 4th pair. Plots of logarithms of length and breadth of successive primordia from the 4th fall along straight lines for ovate leaves and linear, so that the rates of change of leaf dimensions are uniform. The 45° slope of the ovate plots stress the fundamental constancy of the leaf shape, while the slope of the linear series at up to 70° shows how the length is increasing at a higher rate than the breadth. Venation differences in the two types of leaves are first seen at about the 4th primordium.

2. Under conditions of continuous illumination and a temperature of 25° C., the first sign of change in the submerged ovate-crowned shoot is the lengthening of the 7th or 8th primordium, visible within 43 hours. It appears likely that under these conditions, primordia fail to be initiated, but the youngest of those already in existence form typically linear leaves, in some cases with one vein.

3. When ovate-crowned shoots are submerged in summer in a tank in the open under normal conditions of temperature and illumination no cessation in the initiation of leaf primordia occurs. In both submerged and floating crowns some 3 leaves are initiated in 8 days. The submerged shoots elongate much more than the shoots whose crowns are floating, the difference in elongation occurring in those internodes which formed part of the crown before submergence. The greater elongation of the submerged shoot is a result partly of the elongation of more internodes from the crown and also of a greater elongation of each of those internodes. As a result the submerged crown at the end of the experiment contains some 9 nodes as against some 13 in the floating crowns. Whether this is a temporary feature or a consistent one cannot be stated. In winter, however, floating and submerged crowns contain an equal number of nodes.

4. Plots of the logarithms of length and breadth of successive leaves of the submerged shoots in (3) show a straight line characteristic of linear leaves for the 4th, 5th, and possibly 6th primordia. Since 3 primordia were formed in the duration of the experiment, there appears to be a lag between the submergence of the crown and the initiation of the mechanism which alters the shape of the leaves.

5. Small ovate leaves are induced on linear-crowned shoots of *Callitriche intermedia* when these are submerged in 30 per cent. sea water and allowed to remain in the medium upwards of 11 days. These leaves resemble normal ovate leaves in form and in the shape of the epidermal cells. The stomate frequency approximates to that of a normal ovate leaf of the 8th node (immature at this stage). The form of the transitional leaves, with ovate characteristics at the base, is seen to follow partly from the method of maturation of the leaf, which matures from the leaf tip towards the base. The induced ovate leaves are 1-veined, as are the leaves of axillary buds of ovate-crowned

shoots immersed in 30 per cent. sea water. It is suggested that this indicates that the form of the leaf and the venation are characters which are controlled independently.

6. The apices of shoots bearing the induced ovate leaves are vegetative. Associated with this feature is the marked development of axillary buds with ovate leaves.

7. The induction of the ovate leaves is suggested to occur as a result of the increased osmotic pressure of the surrounding medium following immersion in the diluted sea water. The osmotic pressure increase may directly affect the primordial form through turgor changes. A more likely suggestion is that the change in leaf form is controlled via an auxin mechanism which responds to fluctuations in the turgor pressure of the leaf cells.

Acknowledgements

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Appendix Table I

Crown Submergence Periods (*Leaf Development Expt. a*)

Date.	Time.	L/S crown in Pl. XIII.	Number of internodes (each exceeding 1 mm.).
Jan. 5	12.30 p.m. (start)	A1	5
	3 p.m.		
	11 ,,		
Jan. 6	7 a.m.	—	6 to 7
	3 p.m.	—	
	11 ,,	—	
Jan. 7	7 a.m.	A2	7 to 8
	3 p.m.		
	11 ,,	A3	
Jan. 11	11 ,,	A4	13 to 14

Appendix Table II

*Comparison of Primordial Length Originally and at 43 Hours** (*Leaf Development Expt. a*)

Original crowns.		Crowns after 43 hours (A2 of Table I).	
Primordial number.	Average length (mm.).	Primordial number.	Average length (mm.).
7	0·86	7	0·94
8	0·91	8	1·26
8	1·00	8	1·17
8	1·08	8	2·00
	Average 0·96		Average 1·34

* Comparison between sections where youngest primordia were of the same order of development.

Appendix Table III

Shoot Length and Number of Nodes at August 20 (Leaf Development Expt. b)

Crowns submerged (G).*	Nodes between crown and marker.	Crown nodes.	Total nodes.	Base of crown to 3rd node below marker (mm.).	Base of crown to node above marker (mm.).	Length of 3 internodes (marker and 2 below) (mm.).
1	7	9	16	21·4	15·3	6·1
2	5	8	13	9	4·4	4·6
3	5	7	12	4	1·6	2·4
4	6	10	16	17†	11·8	5·2†
5	4	10	14	18·3	13·2	5·1
6	7	9	16	18·1	14·2	3·9
7	6	9	15	17·2	14·3	2·9
8	8	9	17	16·4	12·5	3·9
9	8	9	17	19·0	13·9	5·1
10	8	10	18	15·8†	12·2	3·6†
	64	90	154	123·4	113·4	34·0
	Average 6·4	9	15·4	15·4	11·3	4·3
Crowns floating (F).*						
1	2	12	14	5·5	1·1	4·4
2	1	14	15	6·5	0·6	5·9
3	3	14	17	3·8	1·5	2·3
4	3	12	15	3·7	1·2	2·5
5	2	13	15	6·0	1·0	5·0
6	3	12	15	6·5	1·8	4·7
7	2	14	16	3·4	0·9	2·5
8	3	12	15	6·7	1·4	5·3
9	1	12	13	4·7	0·7	4·0
10	2	12	14	4·8†	1·0	3·8†
	22	127	149	46·8	11·2	36·6
	Average 2·2	12·7	14·9	5·2	1·1	4·1

Number of crown nodes in naturally occurring material

Ovate crowns Aug. 12 (E).*	Linear crowns Aug. 18 (H).*	Ovate crowns Jan. 14 (D).*	Linear crowns Jan. 14 (A).*
12	9	16	15
10	10	14	14
11	10	14	12
12	10	13	13
13	—	14	13
12	11	13	15
13	10	13	14
12	11	13	15
12	—	14	17
14	—	13	13
121	71	137	141
Average 12·1	10·1	13·7	14·1

The marker indicated the base of the crown at the beginning of the experiment.

* Letters identifying shoots in Text-figs. 1 and 2.

† Only two nodes preserved below marker (values not included in average).

— Crowns incomplete.

Appendix Table IV

Experimental Induction of Ovate Leaves in C. intermedia (Series 1)

Serial number of shoot, ovate (*o*) linear (*l*).	Number of internodes.*	Length of shoot (cm.).†	Shoots immersed in:	Condition of crowns at July 30.	Notes on shoot elongation by July 30.
1 *o*	3	4		Dead	slight
2 *o*	3	4·5		*o*	,,
3 *o*	4	4·5		*o*	,,
4 *l*	4	4·4	30% sea water	*o*	,,
5 *l*	9	4·4		*o*‡	,,
6 *l*	10	4·3		*o*‡	,,
				Dead	,,
7 *o*	4	4·6		*l*	medium
8 *o*	4	5·3		*l*	marked } about
9 *o*	5	5·4	tap-	*l*	marked } 8 cm.
10 *l*	6	4·3	water	*l*	slight
11 *l*	4	4·7		*l*	,,
12 *l*	4	4·5		*l*	,,

* Internodes recorded when exceeding 1 mm.
† Measured from base of shoot to tip of leaf projecting farthest above crown.
‡ With ovate-leaved axillary buds.

Appendix Table V

Experimental Induction of Ovate Leaves in C. intermedia (Series 2). Records of Evaporation Losses from Test Tubes. (All weights in gm.)

Date.	Serial number of tube.	Weight of beaker +rod +shoot +tube.*	Weight of beaker +rod +shoot +tube +liquid.	Weight of liquid.	Loss in weight.	% loss.	Concentration of the diluted sea water (%).
Apr. 8	A2	96·43	151·37	54·94	—	—	—
	B2	90·17	129·93	39·76	—	—	—
	C3	79·71	124·76	45·05	—	—	—
Apr. 15	A2	—	148·58	—	2·79	5·1	32
	B2	—	128·01	—	1·92	4·8	—
	C3	—	122·01	—	2·75	6·2	32
Apr. 19	A2	—	147·02	—	4·35	8·0	32
	B2	—	127·08	—	2·85	7·2	—
	C3	—	120·46	—	4·30	9·6	33
Apr. 25	A2	—	145·37	—	6·0	10·9	34
	B2	—	126·00	—	3·93	9·9	—
	C3	—	119·13	—	5·63	12·5	35
May 3	A2	—	143·29	—	8·08	14·7	35
	B2	—	124·61	—	5·32	13·3	—
	C3	—	117·61	—	7·15	16	36
May 12	A2	—	142·32	—	9·05	16·6	36
	B2	—	123·91	—	6·02	15·1	—
	C3	—	116·85	—	7·91	17·6	36

* The test-tube was placed in a beaker for convenience in weighing.

Appendix Table VI

Experimental Induction of Ovate Leaves in C. intermedia (Series 2)

Serial number of shoot, ovate (*o*) linear (*l*)	Number of internodes.*	Vol. of liquid in test-tube (ml.).	Shoots immersed in:	Notes on Shoots at May 12		
				Number of internodes.	Length of shoot (cm.).†	Condition of crown leaves.
A1 *l*	10	55	30% sea water	No growth		10 crowns with ovate leaves. Remaining 5 shoots dead.
A2 *l*	11	55				
A3 *l*	10	55				
A4 *l*	8	60				
A5 *l*	12	60				
A6 *l*	9	50				
A7 *l*	6	55				
A8 *l*	8	50				
A9 *l*	9	55				
A10 *l*	10	45				
A11 *l*	13	45				
A12 *l*	8	40				
A13 *l*	9	40				
A14 *l*	9	45				
A15 *l*	7	45				
B1 *l*	11	60	tap-water	23	10	*l*
B2 *l*	9	40		18	7·5	*l*
B3 *l*	7	45		17	10	*l*
C1 *o*	5	50	30% sea water	dead		*o*
C2 *o*	4	45		7	6§	*o*
C3 *o*	6	45		dead		*o*
D1 *o*	6	55	tap-water	13	10	*l*
D2 *o*	6	40		19	19‡	*l*
D3 *o*	4	45		18	26‡	*o/l*

* Internodes recorded when exceeding 1 mm.

† Original length from shoot base to crown base was 5 cm.

‡ To prevent the crowns of these shoots from reaching the surface, the shoots were wound around the glass rod.

§ Marked development of axillary buds (with 1-veined ovate leaves).

Ovate leaves first recorded on shoots A2, A5, and A14 on April 19.

By May 12, while most of the older leaves of the shoots in 30 per cent. sea water were in a decayed condition and covered with fungi, the induced ovate crowns were, apart from dead crowns, green and reasonably healthy looking. Plants in tapwater were green and healthy looking.

Appendix Table VII

Experimental Induction of Ovate Leaves in C. intermedia (Series 2). Stomatal and Epidermal Cell Frequencies on Adaxial Surfaces of Induced and Normal Leaves. (Each count in an area of 0·0095 mm.² unless otherwise stated)

C1 (*I*=2·1*)		C2 (*I*=2·2) upper half		C2 (*I*=5·2) lower half		C3 (*I*=6·6)	
†stomates.	epidermal cells.	stomates.	epidermal cells.	stomates.	epidermal cells.	stomates.	epidermal cells.
0	14	0	11	0	26	2	27
0	11	0	13	2	31	1	30
0	16	0	13	3	34	3	35
0	17	0	15	3	35	5	24
0	17	0	14	2	29	2	25
0	15	0	17	1	31	1	22
1	20	0	18	2	28	0	20
1	23	2	23	2	29	2	20
0	23	1	24	1	27	0	14
2	33	1	27	0	19	0	11
4	189	4	175	16	289	16	228
Average 0·4	18·9	0·4	17·5	1·6	28·9	1·6	22·8
Per. mm.² 42	1990	42	1840	167	3040	167	2400

C4 (*I* = 5·9)		C5 (*I* = 4·7)		C6 (*I* = 0·6). Count in an area of 0·0306 mm.²	
Stomates.	Epidermal cells.	Stomates.	Epidermal cells.	Stomates.	Epidermal cells.
3	31	1	8	0	16
1	28	1	7	0	15
2	25	1	14	0	12
0	22	2	12	0	14
2	21	1	21	0	24
1	21	2	23	1	19
1	23	2	32	0	18
1	12	2	35	0	20
2	14	2	60	0	17
0	10	0	70	0	25
13	207	14	282	1	180
Average 1·3	20·7	Average 1·4	28·2	0·1	18
Per mm.² 137	2180	Per mm.² 147	2970	3·3	588

Some stomatal indices of other induced ovate leaves

Leaf comparable in form to:	Stomatal index.
C2	4·1
C3	4·7
C3	9·5
C3	6·2
C3	4·3

* and †. See notes on following page.

Appendix Table VII—*continued*

Stomatal and Epidermal Cell Frequencies on Adaxial Surfaces of Mature Normal Ovate and Linear Leaves

(Values are for separate leaves and each value represents the total of ten counts each in an area of 0·0306 mm.²)

Ovate leaf (I = 10·4)		Linear leaf (I = 1·4)	
Stomates.	Epidermal cells.	Stomates.	Epidermal cells.
15	110	1	109
13	119	5	95
10	122	1	89
17	102	2	104
12	120	2	111
10	103	0	109
15	117	0	96
19	127	2	106
11	101	1	105
10	113	1	119
132	1134	15	1043
Average 1·3	11·3	0·15	10·4
Per mm.² 42	369	4·9	340

* Stomatal Index (I) calculated according to Salisbury (1927):

$$I = \frac{S}{S+E} \cdot 100,$$

where S and E are the numbers of stomates and ordinary epidermal cells in a given area.

† Stomatal and epidermal cell frequencies recorded in order from leaf tip to base.

A 1–5. (× 10). Longitudinal sections through crowns of *C. intermedia* shoots (Leaf Development Expt. a).
A 1. Ovate crown.
A 2. After 43 hours submergence.
A 3. After 2½ days submergence.
A 4. After 6½ days submergence.
A 5. Linear crown.
B 1, 2. (× 85). Longitudinal sections through crown bearing induced ovate leaves (B 1) and normal crown bearing linear leaves (B 2).
C 1–4. Transition from linear to induced ovate leaves. Leaves from successive nodes except that a leaf has been omitted between C 1 and C 2.
C 5. Immature ovate leaf (from leaf pair 8).
C 6. Immature linear leaf (from leaf pair 8). (Arrows indicate positions of photomicrographs (D).
D 1–4. (× 240). Photomicrographs of epidermal cells from adaxial leaf surfaces.
D 1. – Leaf C 2.
D 2. – Leaf C 4.
D 3. – Leaf C 5.
D 4. – Leaf C 6. (Orientation of these prints identical with that of the leaves in C.)

Experimental and Analytical Studies of Pteridophytes

XXVIII. Leaf Symmetry and Orientation in Ferns

BY

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With sixteen Figures in the Text

Abstract

An account is given of surgical treatments which may determine the symmetry and orientation of leaf primordia in ferns. In one series small deep tangential incisions, severing the incipient vascular tissue, were made immediately above very young leaf primordia or primordium sites: the primordia developed as leaves in all cases. In the other series two deep and wide tangential incisions were made above a primordium (or site) so as to leave a 'bridge' of intact tissue between it and the apical cell of the shoot. In some of the more actively growing apices buds were formed, even though the shoot apical cell was quite undamaged; relatively inactive apices typically yielded leaves. It is concluded that the orientation and symmetry of a leaf primordium cannot be referred only to the direct action on it of a growth-regulating substance moving basipetally from the apical cell, but rather that these characteristic developments are mediated through the organization and physiological activity of the apex as a whole, the intact apical cell being a central and essential element of the system.

Introduction

On the basis of experimental observations the writer (Wardlaw, 1949) has suggested tentatively that the symmetry and orientation of a leaf primordium in a leptosporangiate fern such as *Dryopteris aristata* (*D. austriaca*) are referable to the distribution of growth in the position in which it originates and possibly to inhibitive effects proceeding basipetally from the shoot apical cell (or apical cell group). A leaf primordium in *Dryopteris* originates as an elliptical outgrowth near the base of the broad apical cone, the tangential component of growth of the subtending sector being large relative to the vertical and radial components. In this position, also, the rate of growth on the adaxial side is considerably less than on the abaxial side, and this difference soon becomes more marked as the latter impinges on the rapidly widening sub-apical region. At an early stage the leaf primordium acquires a large 'two-sided' apical or initial cell as a result of the enlargement of one of its more centrally placed, superficial prism-shaped cells. The orientation of this apical cell is such that the segments produced by its division lie in the tangential plane of the shoot apex, i.e. in the plane of most rapid growth. These segments constitute the marginal meristems from which all the tissues of the primordium are formed. It may well be, as direct morphological observations

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suggest, that the shape and disposition of the leaf apical cell are directly attributable to the relatively large tangential component of growth in the primordium site. Once the two-sided apical cell has been established, the foliar nature of the primordium is irreversibly determined. It thus appears that the orientation, dorsiventral symmetry, and shape of a developing primordium may be referred to the growth activity of the region of the shoot apex on which it originates and to the disposition and activity of its own apical and marginal meristems. The flat, adaxial surface of a primordium, as compared with the well-rounded abaxial surface, is such as to suggest that leaf symmetry and orientation may also be due in part to the localized action of an inhibitive substance on the adaxial side; and indeed, in ferns such as *Matteuccia struthiopteris*, this conception would appear to be borne out by the presence of deep axillary pockets or holes. Moreover, in *Dryopteris*, if the position of the next leaf primordium to be formed, i.e. I_1, is isolated from the shoot apical cell by a wide and deep tangential incision, or if P_1 or P_2 are similarly isolated before they are irreversibly determined as leaves, i.e. by having acquired their two-sided apical cell, the isolated site or primordium develops as a bud, i.e. as a lateral organ of radial symmetry, its rapid adaxial growth being a conspicuous and notable feature (Wardlaw, 1949; Cutter, 1954). In such experiments the tangential incision would tend to eliminate the effects of inhibitive substances moving downwards from the apical cell group. However, a warning that leaf symmetry and orientation may not be accounted for in such a simple and direct manner is given by the observation that if the shoot apical cell is injured, and its further physiological action thereby precluded, the apical meristem may nevertheless give rise to both leaves and buds (Wardlaw, 1950). It thus seemed desirable to ascertain whether the symmetry and orientation of leaf primordia are to be attributed (*a*) to a localized inhibition of growth on the adaxial side by a substance proceeding from the apical cell group, or (*b*) to the regulated growth which is characteristic of the shoot apex as a whole. The purpose of the present paper is to describe some experiments in which these two contrasted hypotheses have been tested.

In relation to the second hypothesis, it may be noted that there is a steady acceleration in the rate of growth from the apex to the base of the apical cone and a rapid acceleration of growth in the region of transition to the sub-apical zone. Thus wide and deep incisions above a primordium, or primordium site, would not only preclude inhibitive effects proceeding from the apical cell group, but would cause radical disturbances to the whole acropetal and basipetal movement of metabolic substances. There is considerable support for the view that the apical cell group is a region of special metabolism, as is also each growth centre which may become a leaf or, if subjected to experimental treatment, a bud. Products of the metabolism of the apical cell group, moving basipetally by way of the prevascular tissue, may exercise a regulative effect on the growth of the apical and sub-apical regions. Thus in the upper part of the apex, where the concentration of a growth-regulating substance produced by the apical cell group will be high, the rate of growth of the

meristem cells may be kept low; but lower down and in the region of transition to the sub-apical zone, where the concentration of the growth-regulating substance will be much lower, growth may be promoted. This hypothetical substance, which might be an auxin or hormone, or a precursor of one or other of these, is likely to be essential in some way to protein synthesis, as Skoog (1954) has recently suggested. An important feature of this conception is that the substance will produce its organizing and morphogenetic effects by the way it affects the growth of individual cells, each of which will react in a characteristic way according to its physiological state, rather than by its action on primordia as such. An alternative view might take the form that the apical group is not a locus of special metabolism, the organization of the apex being due to competition among its constituent cells for the nutrients involved in growth. As these move acropetally from the older regions below, with differential utilization of the several substances at different levels *en route*, the supply will diminish progressively and change qualitatively as the shoot tip is approached. The most satisfying explanation, however, probably lies in a combination of the two views. Thus destruction or isolation of the shoot tip would simultaneously remove both its regulative and competitive activities (see also Wardlaw, 1955).

Experimental Observations

Experimental treatments. Of a considerable and varied series of surgical experiments which have now been carried out, the two most directly relevant to the problems of leaf and bud formation are illustrated diagrammatically in Fig. 1. (The terminology is that now usually employed in studies of leaf inception and phyllotaxis.) In one series small deep tangential incisions, severing the incipient vascular tissue, were made immediately above I_1, I_2, P_1, and P_2. If dorsiventrality in a primordium is due to an adaxial limitation of growth by a growth-regulating substance from the apical cell group, some bud formation might be expected in this experiment. In the other series two deep and extensive tangential incisions were made just above a primordium (or primordium site) so as to leave a 'bridge' of intact tissue between it and the apical cell group. If dorsiventrality is mainly due to a localized adaxial inhibitional effect proceeding from the apical cell group, then leaves should still be formed; but if it is a result of the regulated growth of the apex as a whole (or of the sector in which the primordium is situated), then, in relation to the extensive restriction of both the acropetal and basipetal movement of metabolic substances by the tangential incisions, and the consequential radical disturbance of the normal distribution of growth in the apical cone, some bud formation might well be expected. In both experiments deep incisions were made so as to sever the incipient vascular tissue, Wardlaw and Cutter (1954) having shown that shallow incisions are relatively unimportant in modifying organogenesis.

Experimental results. The results of these two experiments are set out in

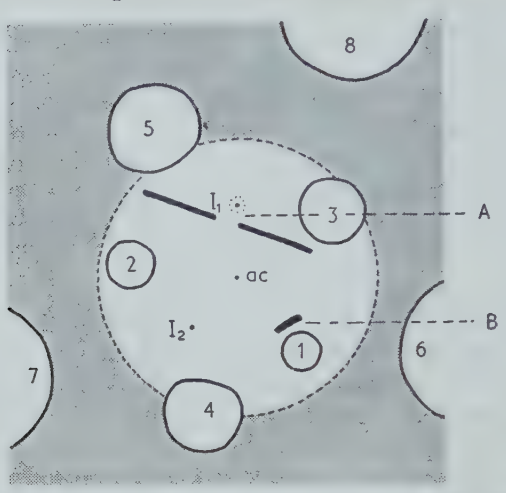


FIG. 1. *Dryopteris aristata.* Apex as seen from above, showing leaf primordia 1–8 and the positions of I_1, the next primordium to be formed, I_2, the next after I_1, and the apical cell *ac*. (A) Two wide and deep tangential incisions have been made above I_1 so as to leave a 'bridge' of intact tissue between the primordium site and the apical cell. (B) A small deep incision has been made on the adaxial side of P_1.

TABLE I

Effect of Small Adaxial Incisions

Primordium.	Leaf formed.	Bud formed.	No development.
I_2	2	—	—
I_1	6	—	2
P_1	9	—	1
P_2	3	—	—
Total	20	0	3

Note: In other specimens, in which the shoot apex had accidentally been slightly damaged, 13 leaves and 6 buds were obtained.

TABLE II

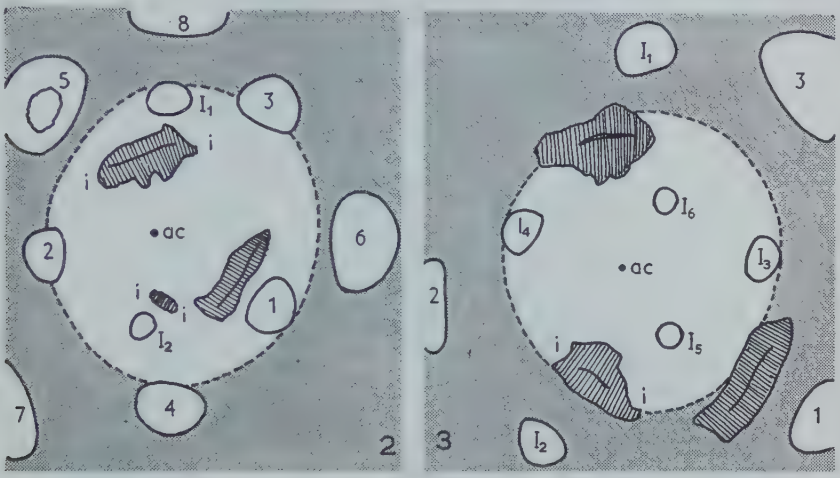
Tangential Incisions with Intact 'Bridge'

Primordium.	Leaf formed.	Bud formed.	No development.
I_2	3	—	—
I_1	7	5	3
P_1	9	2	1
P_2	1	1	—
Total	20	8	4

Note: Where the 'bridge' had been eliminated, i.e. by the two incisions joining up, or where the shoot apex had been damaged, even very slightly, buds were usually formed. Where the 'bridge' was wide, leaves were usually obtained, though occasionally a bud was formed.

Tables I and II, the records relating to specimens in which the apical cell group remained intact and quite undamaged. It was the writer's experience that these experiments are of some delicacy and not easy to perform successfully: many specimens were discarded because of wound damage being transmitted to the apical cell.

Figs. 2–4 illustrate some typical results when small incisions were made above leaf sites or very young primordia. The development of primordia was approximately normal, e.g. they underwent no conspicuous enlargement, and



Figs. 2 and 3. Fig. 2. Camera-lucida tracing of an apex (as seen from above) in which small deep incisions (*i*,*i*) had been made above the axil of a young P_1 and above the site of I_1. With the growth of the apex the incisions have widened out. Leaf primordia have been formed in both instances. At this stage I_2 had appeared and a small incision was made above it (see Fig. 3). *ac*, position of apical cell; 1–8, leaf primordia present at beginning of the experiment; I_1, I_2, new primordia which have subsequently appeared; the light stippling indicates (approximately) the sub-apical region adjoining the apical cone. (Diagrammatic, ×20.) Fig. 3. The same apex as in Fig. 2, after further growth. I_2 has also developed as a leaf primordium. I_3–I_6 have now appeared. (×20.)

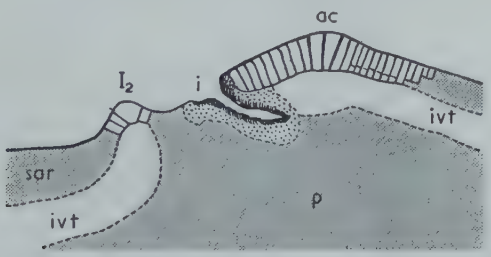
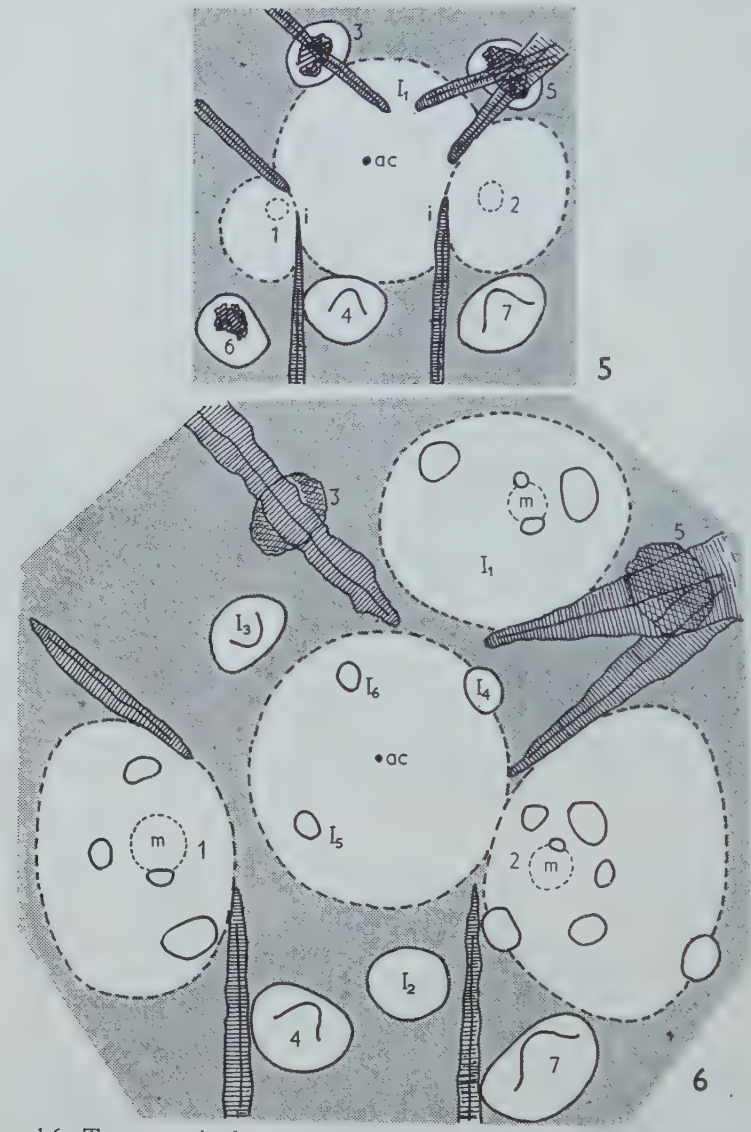


Fig. 4. Longitudinal median section of the apex shown in Fig. 3: the section passes through both I_2 which had developed as a leaf primordium, with its large apical cell, and the apical cell, *ac*, of the shoot. The deep incision, *i*, which is seen to sever the incipient vascular tissue, *ivt*, is surrounded by wound tissue; *p*, pith; *sar*, cortex of sub-apical region. (Semi-diagrammatic, ×55.)

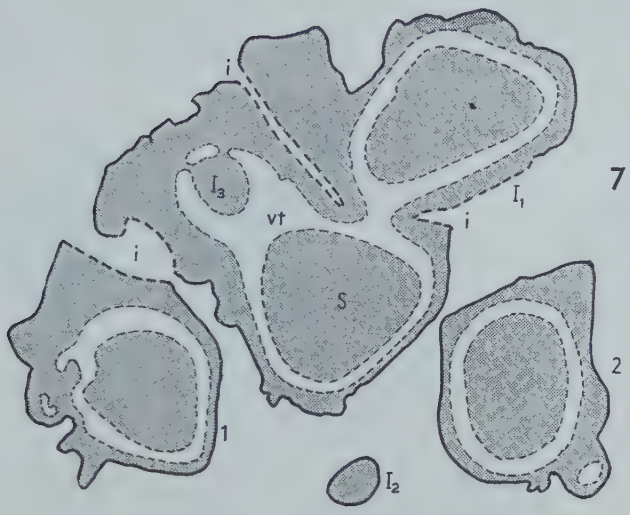
the further growth and morphogenetic activity of the shoot apex were virtually unaltered. Fig. 4 shows that the incipient vascular tissue, which normally extends from the apical cell group downwards, had been effectively interrupted.

In Figs. 5 and 6 the most interesting specimen of the 'bridge' experiment is illustrated. This was apparently a particularly active apex and, soon after the incisions had been made, there was evidence of rapid growth round the



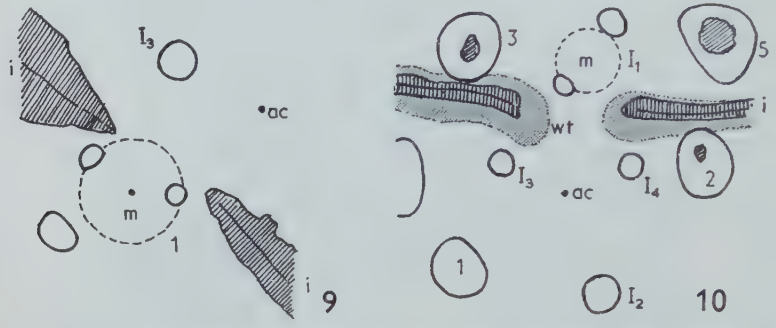
FIGS. 5 and 6. Two stages in the growth of an apex in which tangential incisions, leaving a 'bridge' of intact tissue, had been made above primordia P_1 and P_2 and the site of I_1. (Indications as in previous figures; *m*, apical meristem of induced buds.) Fig. 5. The basal regions of P_1 and P_2 have become greatly enlarged, the original primordium being visible on the top of the tissue mound. Fig. 6. On further growth, it can be seen that large buds have been formed (*m*), each with several leaf primordia. I_2–I_6 have now appeared. (Semi-diagrammatic, ×20.)

basal regions of P_1 and P_2, the original primordial outgrowths being still perceptible on the tops of the considerable mounds of tissue (Fig. 5). Later I_1 developed as a broad swollen region and I_2 as a normal leaf primordium. Still later it was evident that P_1, P_2, and I_1 had developed into large buds, each



FIGS. 7 and 8. Transverse sections of the specimen illustrated in Fig. 6. Cortex and pith, stippled; *s*, axis and stele of main shoot; *i,i*, incisions; *vt*, vascular tissue. Fig. 7 shows that the large solenostelic bud which developed in the I_1 position is in vascular continuity with the main axis, *s*. Similarly, in Fig. 8, the solenostelic buds formed from P_1 and P_2 are in vascular continuity. At this level the pith of bud I_1 is confluent with that of the main axis. (Semi-diagrammatic, ×25.)

with several leaves, while I_2–I_6 had developed as leaf primordia, occupying approximately normal positions on the meristem (Fig. 6). When this apex was sectioned transversely, the foregoing developments were confirmed (Figs. 7 and 8). The 'bridges' were intact and quite wide, and each bud was in



FIGS. 9 and 10. Incised apices with intact 'bridges' have developed buds, *m*, at P_1 and I_1 respectively. In the latter there was a very conspicuous development of wound tissue, *wt*, round the incisions. Lettering as before. (×20.)

vascular contact with the incipient vascular tissue of the apex. The large buds have wide and conspicuous solenosteles. Lower down, below the region of vascular continuity, the piths of the shoot and bud steles constitute an uninterrupted zone of parenchyma.

Fig. 9 shows an apex in which P_1 was transformed into a bud, even though

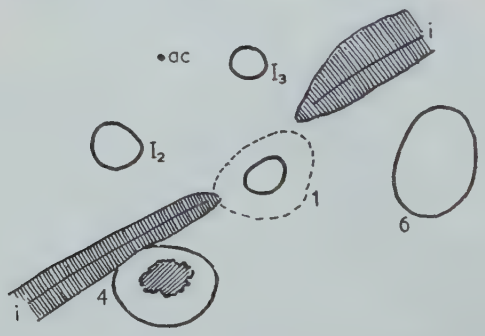
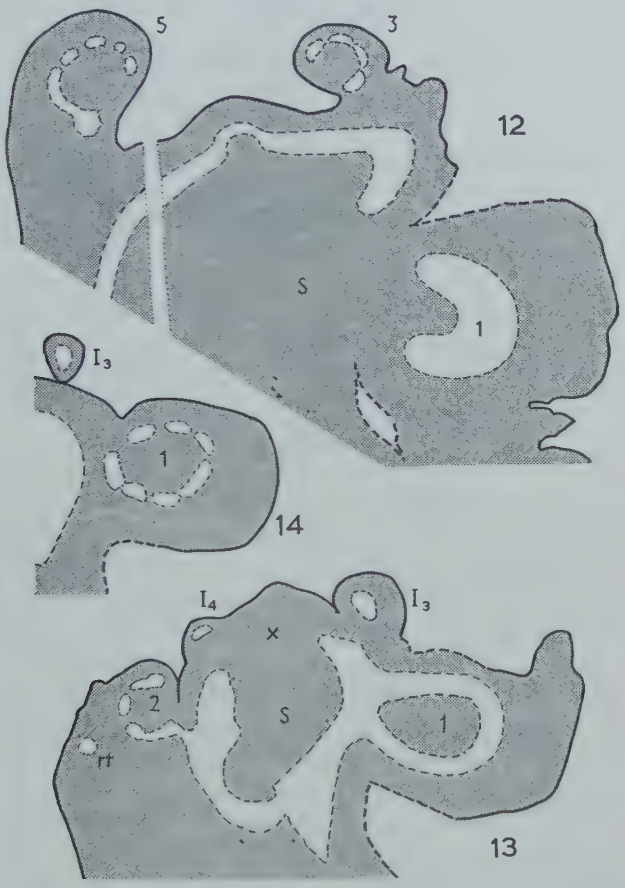


FIG. 11. P_1 has developed into a leaf primordium with a greatly enlarged basal region (see Figs. 12–14). (×20.)

a comparatively wide 'bridge' was present. Fig. 10 illustrates the fact that in apices in which buds were obtained there was rapid and extensive growth and wound-healing in the region of the incisions. In the apex shown in Figs. 11–14, P_1, with a wide intact bridge above, became a considerably and abnormally enlarged leaf primordium. (The possibility that it may already have been determined as a leaf at the time of treatment cannot be completely ruled out.) As the transverse sections show (Figs. 12–14), this primordium, which was

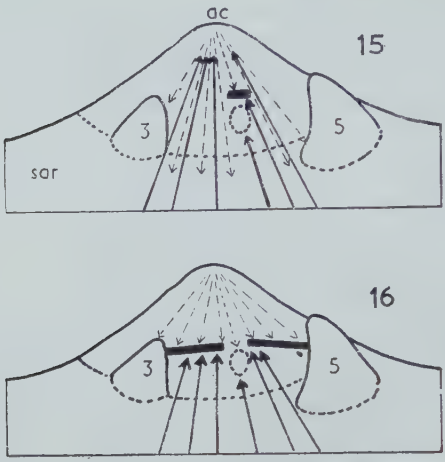
very large relative to older primordia such as P_3 and P_5, had a large and conspicuous leaf trace. Moreover, near its base the contours of the primordium and of its vascular tissue resemble those of a centric or radial leaf.

Lastly, as part of the relevant experimental evidence, it may be noted that



FIGS. 12–14. These show cross-sections, at different levels, of the apex illustrated in Fig. 11. Fig. 13 shows the partly isolated leaf primordium, P_1, at the level where its solenostelic vascular system becomes confluent with that of the main shoot, *s*. (This section is slightly oblique, the cross indicating the approximate position of the apical meristem in sections higher up.) The sizes of P_1 and P_2 may here be compared. Fig. 12, taken a little lower down, enables a comparison to be made of the vascular developments in P_1, P_3, and P_5. Fig. 14, taken above the level of Fig. 13, also shows the almost centric character of the primordium: *rt*, root trace. (×25.)

in apices in which the apical cell had been destroyed, with concomitant induction of buds at I_1, P_1, and P_2, some of the later-formed leaf primordia, e.g. I_3, I_4, &c., were either orientated towards the nearest bud or outwards and downwards, i.e. away from the shoot-tip. This evidence is considered in detail elsewhere (Wardlaw and Cutter, 1955).



FIGS. 15 and 16. Diagrammatic representation of the apical cone with distal apical cell, *ac*, and the wide sub-apical region, *sar*. Two primordia P_3 and P_5, with the I_1 position in between, are shown. The extent to which the two types of incision might interfere with acropetal and basipetal metabolic gradients is suggested.

DISCUSSION AND CONCLUSIONS

The evidence from the first set of experiments (Table I) is conclusive: a small incision above the axil of a primordium or primordium site has in no instance resulted in bud formation. It thus appears that the slow growth of the adaxial side of primordia and their consequential dorsiventrality are probably not due to the localized action of a basipetally moving growth-regulating substance. It is, of course, conceivable that a growth-regulating substance could pass round the incision to the adaxial side of the primordium, but this does not seem very probable since, even where the incisions were deliberately made rather wide, leaves were still formed.

The evidence from the second series, though less conclusive, is none the less important. In at least some apices (actually 25 per cent. of all primordia and 40 per cent. of the I_1s that grew), bud formation was induced when the apical cell was undamaged and when intact tissue extended between it and the primordium. In those apices in which buds were formed, the experimental region was marked by conspicuous growth activity and the buds were of large size. In some reactive apices, although leaves were formed, these were almost of a centric or radial character. Late season apices, which were relatively inactive, typically yielded leaves. In this experiment the two wide tangential cuts go a long way towards isolating the shoot-tip, i.e. in the experimental sector (Fig. 16) and, as we have seen, marked growth activity may be induced on the adaxial side of primordia, and buds may be formed. On the other hand, as even a cursory inspection will show (Fig. 15), a small incision above a primordium will do comparatively little to modify the normal movement of metabolites in the apex.

As rapid wound healing, with formation of parenchyma and an outgrowth of scales, takes place when the apical cell is damaged and in proximity to the tangential incisions, it may justly be inferred that both the quantity and quality of the nutrients being drawn upwards are different from those utilized in the normal growth of the apical meristem. Extensive scale and parenchyma formation, indeed, are characteristic of the normal growth developments on the abaxial side of actively developing primordia. Furthermore, in apices with a damaged tip, young primordia, already determined as leaves, may be observed facing and curving outwards instead of inwards, in relation to the high rate of growth on their adaxial side. The cumulative effect of the evidence now available is thus to support the view that a wide tangential incision in the meristem, by precluding both the acropetal and basipetal movements of metabolites, &c., leads to a levelling up of the growth rates on the adaxial and abaxial sides of a primordium and, as a result, an organ of radial symmetry may be formed. It is a notable and characteristic feature of such experiments that the induced bud primordium has a large and conspicuous meristem as compared with a leaf primordium.

The experiments described here confirm earlier views that, in the ferns, the apical cell is not only the focal point in the distal meristem but is essential for the continuing regulated growth and morphogenetic activity of the shoot apex. In that context it may also be regarded as the ultimate determiner of the orientation and symmetry of leaf primordia. On the evidence, however, these characteristic developments in nascent primordia are not due to the direct action of growth-regulating substances moving basipetally from the apical cell: it seems more probable that they are mediated through the organization and physiological activity of the apex as a whole, the intact apical cell being a central and essential element of this complex reaction system.

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Interrelation of Scion and Rootstock in Fruit-trees

I. Weights and Relative Weights of Young Trees formed by the Reciprocal Unions, as Scion and Rootstock, of Three Apple Rootstock Varieties: M.IX, M.IV, and M.XII

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With Plate XIV

ABSTRACT

Three apple rootstock varieties, M. IX, M. IV, and M. XII, were combined as scion and rootstock in all nine ways and samples were harvested at intervals over 3 years. Their relative sizes on each occasion and their relative rates of growth between occasions were determined by calculating the ratios between the geometric mean weights. The ratio of the products of these for trees with reciprocal Unlike unions, e.g. IX/XII × XII/IX, to that for trees with Like unions of the component varieties, e.g. IX/IX × XII/XII, was slightly but not significantly less than unity. Composite trees, e.g. IX/XII, resembled in size those of the rootstock type, e.g. XII, more than the scion variety, IX, though this had some influence.

INTRODUCTION

MOST varieties of fruit-trees fail to breed true from seed and must therefore be propagated vegetatively. The majority are difficult to establish on their own roots, and are therefore normally grafted as scions either on seedlings or on vegetatively raised rootstocks. The resulting composite tree has leaves, blossoms, and fruits of one genetic constitution, roots of another; in apples the stems are usually mainly of the scion variety, but the rootstock may contribute a considerable portion under some methods of propagation and in other tree fruits.

Simple trees of the component varieties have each their own inherent rates and patterns of growth, resulting in the size and form characteristic of the variety, though this may be modified within limits by the environment. How far the component parts in a composite tree will each maintain its own varietal growth characteristics will depend on how far these are dictated by the genetical composition of the part and how far they are the outcome of influences emanating from, or through, neighbouring parts. Students of growth and form in animals are largely concerned with analogous problems of internal regulation, and it is of interest to note that they use grafting as one of their principal tools.

Scion and rootstock are thus parts of each other's environment, and it is generally recognized that each has some influence upon the growth of the other: there is no unanimity of opinion, however, as to which is the dominant

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partner. At East Malling the rootstock has been found to have a profound effect upon the scion (Hatton, 1923, 1935)—though scion may have some effect upon the root (Amos, Hatton, and Hoblyn, 1930)—and the use of clonal rootstocks has become the accepted practice in England, first to eliminate the variability that arises from the use of mixed seedlings of different inherent rates and habits of growth, and secondly because of the opportunity it provides to control tree size and performance by the use of the appropriate rootstock. Striking differences in size of scion due to inherent differences in a range of rootstocks, raised originally from seed, have been recently demonstrated (Preston, 1954). A résumé of rootstock/scion relations is given by Tubbs (1951).

Some workers, on the other hand, have reported the scion to have a marked effect on the growth and habit of the rootstock (Swarbrick and Roberts, 1927; Tukey and Brase, 1933), especially when the whole of the stem is contributed by the scion variety: they have sought to use this scion dominance as a means of obtaining uniformity in trees grafted on to mixed seedlings. Though the necessity for raising rootstocks vegetatively would thus be eliminated, the power to control tree size would be lost; where the scion is dominant it will dictate the size and behaviour of the tree. Moreover, the risk of scion-rooting further prejudices control (Hatton and Bagenal, 1933). It should also be noted here that at East Malling the rootstock has been found to be dominant even when the scion has been grafted on to piece roots (Beakbane and Rogers, unpublished; Hatton, 1938).

It is evident that further understanding of the causes of the reciprocal influence of scion and rootstock is required if they are to be fully utilized in a more efficient control of tree size and performance. A series of investigations, started with this object several years ago, has been briefly described in a recent report (Vyvyan and Maggs, 1954). The experiment now reported was planned to provide further knowledge on three problems. (1) To what extent is the growth rate and resulting size of a young composite tree the resultant of the growth rates of the component varieties; e.g. if we make reciprocal grafts A/B and B/A between the varieties A and B, will the sum of the weights of the composite trees A/B and B/A tend to be the same as that of simple trees of the two varieties A and B? (2) Which will be the dominant partner, rootstock or scion, e.g. which will A/B resemble most, A/A or B/B? (3) To what extent are the ratios between parts, such as root and stem, that are found in simple trees, maintained in young composite trees where the parts concerned are of different genetic constitutions, and how far can the mechanisms responsible for these ratios be held responsible for the observed reciprocal influences of scion and rootstock?

For this purpose three apple rootstock varieties were used and were combined as scion and rootstock in all nine possible ways. The trees were weighed at planting and the course of growth was followed over three seasons by digging up samples at intervals, and determining the weights of the trees and their parts.

The present paper will be concerned mainly with the first two questions mentioned above and will deal with only the non-deciduous portions of the tree—the stems and the roots. The third question—the ratio between parts, including that of leaf weight to the rest of the tree—will be examined in the second paper in this series.

Materials and Methods

Varieties used. When examining the relative effects of scion and rootstock on tree growth and size, it is important to use the same varieties in both capacities.

Three rootstock varieties—M.IX, M.IV, and M.XII—were chosen for ease of propagation, and were combined as scion and rootstock in all nine possible ways—IX/IX, IV/IX, XII/IX; IX/IV, IV/IV, XII/IV; IX/XII, IV/XII and XII/XII.

The dwarfing rootstock M.IX usually produces a small tree and M.XII is one of the most vigorous clonal rootstocks. The third rootstock variety, M.IV, was included because Hatton (1923) found it behaved in a rather unusual manner, producing a larger tree, when worked with a scion of a different variety, than would be expected from its behaviour when grown as a simple unworked tree.

Unworked, unbranched, rooted shoots from the stool-beds were used. They were weighed individually before planting, but were not cut back or trimmed in any way. They were planted in March 1936, budded with the appropriate scion in August 1936, and cut back to these buds in April 1937, whilst still dormant.

Lay-out of experiment. There were six experimental blocks, each consisting of three rows of 56 trees; one row at random for each rootstock variety. Owing to the shape of the available plot of ground, the rows were arranged in pairs, end to end; thus rows 1 and 2 formed a continuous run of 112 trees and rows 1, 2, and 3 constituted block A.

When the scion buds were inserted in August 1936 each row was divided into three segments of 18 or 19 trees, one segment being chosen at random for each variety of scion. It was planned to take one tree from each segment at each harvest. In the statistical analysis of data from such harvests there would be two experimental errors: one between rows within blocks and appropriate for examining the differences between trees on the three rootstocks, and the other between segments within rows appropriate for examining the differences between scions and the interactions between scion and rootstock.

History of experiment. Samples of each kind of tree were harvested at the time of budding (August 1936), time of cutting back (April 1937), in the middle of the scion maiden year (August 1937), and of its second year (August 1938) and in April 1939. On each occasion the fresh and dry weights of each sampled tree, and of its parts—leaves, stems, and roots—were obtained, and certain other measurements were made. The weights of a further large sample of

trees of each kind were obtained in April 1939, when the experiment was ended.

At the first harvest, at the time when the scion buds were inserted and before they could have exerted any influence, only 6 trees per rootstock variety were taken; one at random from each row. The second harvest, taken at the time of cutting back in April 1937, consisted of 54 trees; one from each segment. Before selecting the trees for the harvest due in August 1937 a census was taken of the number of surviving trees in each of the 54 segments. In most segments there were 10 or more available, but in a few the numbers were low, owing to the death of the stocks or failure of scion bud in the initial phase of the experiment. In three segments there were only 5 suitable trees and in two segments there were only 3. This meant that only three further harvests of one tree from each segment would be possible. It was decided therefore to spare these few surviving trees for later harvests, when block effects might become more pronounced, and to substitute on this occasion trees from other segments where the numbers of survivors were large. The harvests collected in August 1938 and April 1939 were according to plan, with 1 tree from each of the 54 segments.

By April 1939 some of the kinds of tree had become rather large and were now too closely spaced to allow unrestricted growth for a further season. It was therefore decided to terminate the experiment and to embody the lesson learnt from it in a plan for a new experiment on the same general lines; a project that could not be carried out until after the war. All the remaining trees were dug up with as much root as could be recovered without complete excavation. All abnormal trees were rejected and the remainder of each scion-rootstock combination were laid out in a row and arranged roughly in order of size, by eye judgement. The centre tree of the row, and the two at mid-distances between the centre and the ends of the row, were selected as typical trees of that scion-rootstock combination, and photographed (see Pl. XIV). Twenty or more of the remaining trees, selected at intervals along the row, were cut up and weighed. For convenience in the statistical analysis of the data the number for each scion-stock combination was reduced to a random 20 where more than that number had been weighed. As the process of cutting up and weighing the trees of this large sample took a considerable time, they had more time to dry out than those of the routine samples; their weights will therefore be referred to as 'air-dry', though, strictly speaking, this may be rather a misnomer, as the weights had probably not come into complete equilibrium with the atmosphere.

Statistical treatment of data. Analyses of variance were carried out in the usual manner on the values for the fresh and dry weights of the various organs at the successive harvests. A parallel series of analyses was also carried out on the logarithms of these values as Pearce (1943, 1953) has shown that this is often desirable.

There were two possible sources of variation whose effects on the standard errors could be at least partially estimated and eliminated; these were (1)

initial fresh weight of the tree at the time of planting, and (2) a possible linear 'fertility gradient' down the long axis of the plot. These possibilities were tested in the case of each harvest.

Adjustment for initial weight at planting. The co-variance between the initial fresh weights at planting and the final fresh weights of the non-deciduous portion (stems and roots) was determined for the trees of each harvest. The regression coefficient for the first harvest proved significant and the values for the fresh and dry weights of the various organs of the trees sampled then were accordingly adjusted by the usual formula.* As the L test revealed that the variance was heterogeneous even after adjustment, the values for each rootstock variety were adjusted separately.

In April 1937 the regression coefficient was not significant, so no adjustment was required. In August 1937 the general level of the regression was just significant, but, when broken up according to rootstock and scion, the regressions appeared to differ in size and sign in an arbitrary manner that followed no logical plan. The only high value was that for trees of XII/XII, and this was largely due to two trees. The values for this harvest were therefore not adjusted. In 1938 and 1939 no adjustments were required as the regression coefficients did not approach significance.

Adjustment for 'fertility gradient'. The long, narrow shape of the plot and of the experimental blocks suggested the possibility of considerable differences in soil fertility, within the blocks, whose effects would not be eliminated by the removal of variance due to block. As each array of 112 trees was divided into six segments of the same size, the whole plot was, in effect, so divided. The convention was followed of numbering these segments from north to south with the serial numbers 1 to 6. The co-variance between the fresh weights (of the non-deciduous portion) of the trees of each sample and the serial numbers of the segments in which they were situated, was calculated; this was found to be significant in the August 1938 and April 1939 harvests, but not for the earlier harvests or for the large final harvest of residual trees. The values for the two harvests concerned were accordingly adjusted. It should be noted that the effects of variation in soil fertility has only been adjusted, by these means, in so far as it can be represented by a linear gradient from north to south of the plot.

The co-variance between the logarithms of the final fresh weights and (1) the logarithms of the weights at planting, and (2) the serial number of the segments, were worked out in the same way, but none of the values was significant and no adjustment was necessary.

Interpretation and Presentation of the Results

The use of logarithmic transformation of data relating to weight and size in fruit-trees has been recommended by Pearce (1943, 1953) on statistical grounds, because it often allows of a more precise evaluation of the results.

* $\bar{y}' = \bar{y}_1 + b(\bar{x}_1 - \bar{x}_0)$, where $\bar{y}'_1$ is the required adjusted value of the mean weight at lifting, $\bar{y}_1$ the unadjusted mean, $\bar{x}_1$ the initial fresh weight of the sampled trees, $\bar{x}_0$ the mean initial fresh weight of all trees of that kind, and b is the regression coefficient, Sx_1y_1/Sx^2_1.

There may, however, also be sound biological reasons why the use of logarithms leads to greater precision. Organisms, especially when young, often tend to increase in weight, under equal conditions, in proportion to their own weight. That is, the *relative* rather than their *absolute* rates of growth tend to be alike. This would not of itself be sufficient to turn a distribution that was initially normal into one that was log-normal and therefore calling for logarithmic transformation. For if the distribution were normal, and every unit doubled in size, they would all have grown at the same relative rate but the distribution would still be normal although the range, mean, and standard error would all have been doubled. A tendency towards a log-normal distribution might arise, however, owing to the nature of the relative growth rate and its probable relation to some of the factors that may affect it. The 'Efficiency Index' (Blackman, 1919) or 'Relative Growth Rate' is best defined (Briggs, Kidd, and West, 1920) by the equation $R = 100\ (\log_e W_2 - \log_e W_1)/(t_2 - t_1)$, where W_2 and W_1 are the weights at time t_2 and t_1 respectively, $(t_2 - t_1)$ the time interval, e is the base of natural logarithms, and R is the required value of the relative growth rate. It is not difficult to imagine circumstances under which this rate might be influenced directly by some factor, such as a nutrient in the soil, whose concentrations might be distributed normally, in the statistical sense. Under such circumstances the values of $\log_e W_2$ would become normally distributed, as may readily be seen if we make $W_1 = 1$, and $\log_e W_1$ therefore $= 0$.

There are also other related reasons why the use of logarithms is of value. When dealing with the effect of factors, such as rootstock, upon tree size, it is the relative differences in size, rather than the absolute differences, that are of interest. It would be manifestly absurd to suggest that trees on two rootstocks differed by some constant amount, such as 100 g., and to claim that this was a constant defining the differences between the two stocks, irrespective of tree size or age. This difference might be important in maiden trees, but would be negligible in mature trees of vastly greater weight, even if statistically significant. It would be reasonable, perhaps, to postulate that the *ratio* between the weight of a tree on one rootstock and that on another should be a constant; to suggest, for example, that a tree on the one might tend to be twice as heavy as that on the other, irrespective of age or size.

If the weights are denoted X and Y respectively, it is the ratio X/Y rather than the difference $X - Y$ that is of interest. Given the arithmetic mean values of X and Y and their standard errors, it is easy to calculate the value of $X - Y$ that will differ significantly from zero at a required level of probability, but it is not possible to estimate from these the value of the ratio that would differ significantly from unity, unless each value of X can be legitimately paired with a particular value of Y so as to provide a number of separate estimates of the ratio. If, however, the analyses are done on the logarithms of the observed values (Pearce, 1953) the mean values of these, which we may denote $\bar{x}$ and $\bar{y}$, when transformed back, become the geometric means $\bar{X}'$ and $\bar{Y}'$ of the observed values; their difference $(\bar{x} - \bar{y})$ becomes the ratio between

the geometric means ($\bar{X}'/\bar{Y}'$), and the significant difference becomes the value for this ratio that would differ from unity at the required level of significance.

If $\bar{X}'_1/\bar{Y}'_1$, $\bar{X}'_2/\bar{Y}'_2$, $\bar{X}'_3/\bar{Y}'_3$ are the ratios between the geometric means of X and Y at times t_1, t_2, t_3, it is possible in this way to determine whether the accumulated effects of the two rootstocks on the relative weights of the trees have been significantly different *up to* the times concerned, but these values do not of themselves necessarily show *during* which of the intervening periods the significant difference in fact occurred. If the ratio for time t_2 is significant and that for time t_1 is not, it is safe to assume that the effects of X and Y were different during the period (t_2-t_1). If the ratios for t_2 and t_3 are both significantly different from unity, there may have been a significant difference in effect during the period (t_3-t_2) or there may not; for it may merely mean that the significant ratio, established before t_2, has been maintained unchanged. To determine which of these possibilities is the more probable, the two ratios must be compared.

If the ratio between ratios $(\bar{X}'_3/\bar{Y}'_3)$: $(\bar{X}'_2/\bar{Y}'_2) = A$, it is required to know if A differs significantly from unity. This value is obtained, by back-transformation, from the differences between the differences of the logarithms; it represents the equation $(\bar{x}_3-\bar{y}_3)-(\bar{x}_2-\bar{y}_2) = a$. The significance of the difference of the quantity a from zero can be determined in the usual way from the standard errors of the four quantities on the left side of the equation. When transformed back, this significant difference will become the value of A that would be significantly different from unity.

If the value is not significant, it suggests that $\bar{X}'_3/\bar{Y}'_3 = \bar{X}'_2/\bar{Y}'_2$ and, consequently, $\bar{X}'_3/\bar{X}'_2 = \bar{Y}'_3/\bar{Y}'_2$, and that the relative weights of trees on the rootstocks have not changed significantly from occasion to occasion and their relative growth rates during the period have not been significantly different.

In this paper the arithmetic means of the weights will be given in every case, since this has been the normal method of presentation of results in the past, but they will be supplemented in most cases by the ratios between the geometric means and by the ratios between these ratios.

Growth of the Three Varieties

The arithmetic means of the fresh weights of the non-deciduous portion (stems and roots) of the trees of the three varieties, on successive occasions from the time of planting (March 1936) until the final harvest (April 1939), are given in the upper part of Table I. Standard errors and significant differences were calculated but are omitted to save space.* The relative weights for each pair of varieties are shown by the ratios between their geometric means, presented in the lower part of the table; these represent the differences between the means of their logarithms, after back-transformation (Pearce, 1953). The levels of significance, indicated by asterisks, are based on the significant ratios derived from the significant differences between the logarithmic means. As explained earlier, these are levels the observed ratios

* They will be made available to correspondents if required.

must attain if they are to be considered significantly different from unity—they have been omitted here to save space.*

The ratios between the weights on two successive occasions, again derived from the logarithms, are shown in the upper part of Table II; thus the values in the top left-hand corner show that trees of M.IX, M.IV, and M.XII increased 3·06-fold, 2·83-fold, and 3·68-fold respectively during the period from March to August 1936. The relative performance from occasion to occasion, of trees of the varieties compared, is indicated by the values of the ratios between ratios given in the lower part of this table. Thus the value 1·08 is the ratio between the value 3·06 for M.IX and 2·83 for M.IV; it is also the ratio between the values 1·38 and 1·28 for the ratio IX: IV in Table I.

Initial fresh weights at time of planting. The 336 trees of each rootstock variety were weighed individually to the nearest gramme before they were planted; the mean weights are given in the first column of Table I. These weights represent 1 year's growth from buds on the parent plants in the stool-beds. The weights ranged from 9 to 32, 6 to 37, and 9 to 72 in the three rootstocks, and in the M.IX's and M.XII's the frequency distribution was more or less symmetrical about the mean values, suggesting that most of the shoots harvested had been used. In the M.IV's, however, it was asymmetrical and suggested that a large proportion of the shoots had been rejected as too small: even so the mean weight of the M.IV's was considerably less than that of the M.IX's—this is normally the case with these two rootstocks at this stage.

Growth of 'simple' trees during the rootstock year before scion grew out. The first year after planting may be regarded as the 'rootstock' year. Although scion buds were grafted on the trees in August of that year, only a few, mainly on M.IV, burst that season, and even these made very little growth; they produced at most a little spur bearing a few small leaves. During the season, therefore, the main bulk of every tree and all, or practically all, its leaves were provided by the rootstock variety and it is unlikely that the weights, or changes in weight, were influenced in any way by the insertion of the scion buds in August, except, perhaps, mechanically as a result of wounding and a possible partial interruption of translocation, and this would probably be the same in all rootstocks. The possibility of some stimulus from the buds, however, cannot be altogether ruled out.

During this season, therefore, we may expect the weights and changes in weight to be governed entirely by the rootstock variety and independent of the scion. It should be noted that there is no such 'rootstock' year in bench-grafted trees; here short lengths of scion stem are grafted on to similar lengths of rootstock root, before planting, and all leaves from the start are provided by the scion variety: nevertheless, at East Malling rootstock has been found to be dominant in such trees (Hatton, 1938; Beakbane and Rogers, unpublished).

The mean fresh weights at the time of planting in March 1936, bud-grafting in August 1936, and 'cutting back' in April 1937 are given in the left-hand columns of Table I. The trees were cut back to the scion buds in a

* They will be made available to correspondents if required.

Table I

Fresh Weights of Non-deciduous Portion of Trees of Each Variety during the First Year after Planting and those of Trees with Like Unions (IX/IX, IV/IV, and XII/XII) during the First and Second Scion Years, and the Ratios between the Weights of Each Pair of Varieties

Date	Mar. 1936	Aug. 1936	Apr. 1937		Aug. 1937	Aug. 1938	Apr. 1939
Trees per unit	336	6	6 groups of 3		6	6	6
Time of	Planting	Insertion of scion bud.	Cutting back to scion bud: Before.	Cutting back to scion bud: After.	Scion maiden year.	Scion second year.	End of second scion year.
	Mean weights						
Variety	g.	g.	g.	g.	g.	g.	g.
IX	16	(49	(72	(41	111	465	508
IV	13	(45	(63	(36	64	710	806
XII	33	104	177	90	180	1,326	2,292
	Ratios						
IX: IV	1·28**	*1·38*	*1·21*	*1·15*	1·76**	0·59***	0·63**
IV: XII	0·38***	0·29**	0·35**	0·40**	0·36***	0·50**	0·35***
XII: IX	2·05***	2·44***	2·37**	2·18**	1·57**	3·36***	4·53***

The values for August 1936 have been adjusted by co-variance for initial weights at planting, those for August 1938 and April 1939 have *not* been adjusted for the 'fertility gradient'.

Bracketed weights are not significantly different and ratios in *italics* do not differ significantly from unity.

* $P = 0{\cdot}05$. ** $P = 0{\cdot}01$. *** $P = 0{\cdot}001$.

Table II

The Ratio between the Weight on One Occasion and that on Another for Each Variety, and the Ratio between these Ratios for Each Pair of Varieties

Occasions	Aug. 1936	Apr. 1937 (*before*)	Apr. 1937 (*after*)	Aug. 1937	Aug. 1938	Apr. 1939
Compared	Mar. 1936	Aug. 1936	Apr. 1937 (*before*)	Apr. 1937 (*after*)	Aug. 1937	Aug. 1938
	Ratios					
IX	3·06***	1·52*	0·55***	2·76***	3·54***	*1·25*
IV	2·83***	*1·74*	0·58***	1·82**	10·53***	*1·18*
XII	3·68***	*1·47*	0·50***	2·00*	7·57***	1·68**
	Ratios between ratios					
IX:IV	*1·08*	*0·87*	*0·96*	1·52*	0·34**	*1·06*
IV:XII	*0·77*	*1·19*	*1·16*	*0·91*	*1·39*	*0·70*
XII:IX	*1·20*	*0·96*	*0·90*	*0·72*	2·14**	*1·35*

Ratios in italics do not differ significantly from unity.

* $P = 0{\cdot}05$. ** $P = 0{\cdot}01$. *** $P = 0{\cdot}001$.

single operation in April 1937—no 'snags' were left above the buds for later removal as is often done. There are two sets of values for this date; those *before* cutting-back represent the weights at the end of the 'rootstock' year, those *after* cutting-back the weights at the start of the scion maiden year.

The mean weights for the three varieties were significantly different at the time of planting, and they were still different, with M.XII the largest and M.IV the smallest, in April 1937. In the August and April harvests, however, the differences between M.IV and M.IX were not significant. The ratios between the values for M.XII and those for M.IX and M.IV were significant on each occasion, but that between M.IV and M.IX only at the time of planting—a result in agreement with that found above by analysis of the untransformed data.

The ratios between the weights in March and August 1936 and April 1937 (Table II) show that all three varieties increased in weight at about the same rate during the rootstock year, and most of the increase occurred before August. None of the ratios between ratios was significantly different from unity. The ratio between the two values for April 1937, in the third column of Table II, is a measure of the proportion of the tree that survived the operation of cutting back. The values show that half, or nearly half, the tree was removed —a very significant reduction in weight—but the ratios between ratios show that there were no significant differences between the varieties in the severity of the operation.

Growth of trees with 'Like' unions during the maiden and second scion years. There were no ungrafted trees in the experiment; all were budded in August 1936. A third of the trees, however, received buds of their own variety; these, the trees with 'Like' unions, IX/IX, IV/IV, and XII/XII, are each composed of a single rootstock variety and are the nearest to simple trees of the varieties.

The mean fresh weights for the six trees of each of these kinds, and the appropriate ratios, are shown in the three right-hand columns of Tables I and II. The values for the mean weights given here for 1938 and 1939 differ somewhat from the corresponding values given below in Table III, because the latter have been adjusted for the 'fertility gradient' and these have not. Adjustment increased the differences between the values for the three root-stocks and reduced the standard errors.

The trees of M.IX grew much more slowly than the others. In August 1937 they were heavier than the M.IV's and two-thirds the weight of the M.XII's. By April 1939 they were only about half the weight of the M.IV's and less than a quarter that of the M.XII's. The differences in growth rate and changes in relative weight are brought out clearly in Table II. During the period between April 1937 and August 1937 M.IX made the greatest growth, increasing nearly 3-fold (2·76), M.IV increased less than 2-fold (1·82) and significantly less than M.IX, for the ratio between their ratios (1·52) different significantly from unity. It was during the period August 1937 to August 1938 that M.IX grew so much slower than the others; it increased only $3\frac{1}{2}$-fold while the M.IV's and M.XII's increased more than 10-fold

(10·53) and 7-fold (7·53) respectively. These relations are brought out clearly by the ratios between ratios, which, where M.IX is concerned, have changed their relation to unity since the previous period and have become highly significant: the value 1·52 has changed to 0·34 and 0·72 has become 2·14.

Taking the 3-year period as a whole, the mean weights of the trees of the three varieties increased from 16, 13, and 33 g. at the time of planting to 508, 806, and 2,292 g. in April 1939, in spite of the severe cutting back in April 1937. Thus trees of M.IX increased about 30-fold during the 3-year period, those of M.IV about 60-fold, and those of M.XII about 70-fold. The relative growth rates (R) can be readily calculated from the ratios between the geometric means, given in the upper part of Table II, or more easily from the logarithmic values from which these have been obtained by back-transformation. Expressed on the annual basis, the values for the three varieties for 1936–7 were 154, 159, and 168 respectively and the mean values for the 2-year period 1937–9 were 125, 156, and 162 in the three varieties. The value for M.IX during the 2-year period was significantly less than those for the other two varieties, there were no significant differences during the first period or between M.IV and M.XII in the second period.

TABLE III

Fresh and Dry Weights of Non-deciduous Portion of Trees of Each Scion-rootstock Combination during the First and Second Years of Scion Growth

	Fresh weights.			Dry weights.		
	Aug. 1937.	Aug. 1938.	Apr. 1939.	Aug. 1937.	Aug. 1938.	Apr. 1939.
	g.	g.	g.	g.	g.	g.
IX/IX	111	379	483	47	171	251
IV/IX	86	470	406	35	210	211
XII/IX	81	509	612	33	214	317
IX/IV	90	552	667	37	242	331
IV/IV	64	710	806	26	310	417
XII/IV	84	626	1,201	33	255	603
IX/XII	158	1,056	1,321	64	463	680
IV/XII	139	953	1,420	57	410	728
XII/XII	180	1,356	2,318	71	586	1,197

The values for 1938 and 1939 have been adjusted by co-variance for the 'fertility gradient' from north to south of the plot.

GROWTH OF THE TREES OF DIFFERENT SCION-ROOTSTOCK COMPOSITION

Mean weights of the nine kinds of tree. The mean fresh and dry weights of the non-deciduous portion (stems and roots) for each of the nine scion-rootstock combinations are given in Table III. The values for 1937 required no adjustment, but those for 1938 and 1939 had to be adjusted by co-variance for the 'fertility gradient'.

The corresponding values for the air-dry weights for the final sample of 180 residual trees, taken in April 1939, are given in Table IV, where they are arranged in a 3×3 square to correspond with the photographs of typical trees of each kind, shown in Pl. XIV.

Standard errors have been omitted from Table III, to save space.* A separate one would have been required for every value in the table, for in 1937 the variance was heterogeneous and could not be pooled for the comparison of two values in the table, and in 1938 and 1939 it had been adjusted. Except in the case of trees with Like unions, which has been dealt with above, differences between two values are of no particular interest, as more than one factor will be involved. The general significance of these various factors is indicated by the analyses of variance that will be described below.

TABLE IV

Mean Weights of the Trees of Each Scion-rootstock Combination in the Final Harvest of Residual Trees, arranged to show Rootstock and Scion Effect and for Comparison with the Photographs (Pl. XIV)

		Variety used as Rootstock			Scion Effect Mean for trees with same scion		
		IX.	IV.	XII.	Scion.	Mean.	%*
		g.	g.	g.		g.	
Variety used as Scion	IX	404	544	1,357	IX/–	769	85
	IV	397	709	1,274	IV/–	794	88
	XII	537	882	2,011	XII/–	1,143	127
Rootstock Effect	Rootstock Mean	–/IX 446	–/IV 712	–/XII 1,548	—	902	—
Mean for trees with same rootstock	%*	49	79	172	—	—	100

* Mean weights expressed as percentages of the general mean.

Certain general trends in these tables, however, are of interest. Trees on M.XII were always the heaviest, and in 1938 and 1939 those on M.IV were heavier than those on M.IX. In 1938 and 1939, scion variety seemed to have some effect; scions of M.XII gave the largest trees on every rootstock—except on M.IV in 1938.

The fresh and dry weights on each occasion follow the same general trend as might be expected; but the increase in dry weight from August 1938 to April 1939 was relatively much greater than that in fresh weight. It is clear that the stems and roots, between them, contained a much higher percentage of moisture in August of both years, when the trees were in active growth, than in April 1939, when they were dormant. Loss of moisture between August 1938 and April 1939 masked in the fresh weights much of the increase revealed by the dry. The fresh weight of the IV/IX trees was actually less in 1939 than in 1938.

In Table IV and Plate XIV, trees on the same rootstock (e.g. IX/IX, IV/IX, and XII/IX) are in the same column, and those with the same scion (e.g. IX/IX, IX/IV, and IX/XII) are in the same horizontal row. The trees with

* They will be made available to correspondents if required.

Like unions lie on the diagonal from the top left to the bottom right corners. It is very evident, from this table and plate, that rootstock had a much greater effect than scion variety in determining tree size.

Values grouped to show effect of various factors on growth. By grouping the values in various ways, and determining the variance between the mean values for these groups, the total variance between the kinds of tree was broken up into portions associated with certain factors that might influence tree weight. The significance of the variance associated with each factor was estimated by calculating the ratio (F) between it and the appropriate value for the mean square error. Parallel analyses of variance of this type were carried out on the fresh weights and the dry weights and on their logarithms. The values of F

TABLE V

Grouping of the Nine Kinds of Tree for the Partition of Interaction between Scion and Rootstock, with the Appropriate Mean Weights for the Final Harvest as an Example

	Nature of union			Union Factor	
	Like	Unlike			
	$L.$	U_1	U_2	Varieties combined.	Mean.
	IX/IX	IV/XII	XII/IV	IV & XII	853
	IV/IV	XII/IX	IX/XII	IX & XII	868
	XII/XII	IX/IV	IV/IX	IX & IV	984
	g.	g.	g.		
Consistency Factor	—	785	879	—	—
Equivalence Factor	1,041	832		—	902

so found, and their levels of significance, are shown in Tables VI and VII, which deal respectively with the observed values and with their logarithms.

The manner in which the variance was partitioned will be made clear by reference to Tables IV and V, where the mean values for the final harvest have been grouped in two different ways. In Table IV the three values in any one column are all for trees on the same rootstock, and each variety occurs once, and only once, as scion; the three mean values —/IX, —/IV and —/XII, with two degrees of freedom, thus provide the valid estimate of *rootstock effect*. In the same way, the three values in any one horizontal row have the same variety as scion, and each variety occurs once, and once only, as rootstock; the three mean values, IX/—, IV/—, and XII/— provide the valid estimate for *scion effect*. The remaining four degrees of freedom represent interaction between scion and rootstock and can be legitimately subdivided as shown in Table V; here the nine values have been rearranged so that each variety appears once as scion and once as rootstock in each column and in each row, so that the mean values of the columns and rows will be independent both of rootstock and scion and be influenced solely by their interactions.

In each horizontal row of Table V, one variety is represented by its Like union, e.g. IX/IX, and the other two by their reciprocal Unlike unions, e.g.

IV/XII and XII/IX. With regard to the columns, the first (L) contains the three values for Like unions—IX/IX, IV/IV and XII/XII—the other two columns (U_1 and U_2) the values for Unlike unions. The difference between the mean value for column L and that for the other two columns provides an estimate, so far as these varieties are concerned, of what will be termed here the '*Equivalence Factor*' (E). This indicates how far the mean weights of trees with reciprocal Unlike unions tend to be the same as those for Like-union trees of the component varieties. Clearly each pair of varieties will have its own value of E; for example that for M.IX and M.IV can be found from the equation $(IX/IX+IV/IV)-(IX/IV+IV/IX) = 2E_{ix.\,iv}$, where IX/IX, &c., are the mean values for the four kinds of tree and $E_{ix.\,iv}$ is the required value of E. The general value of the Equivalence Factor, derived from the mean values for the columns in Table V, is the mean of these three values of E. The differences between the mean values for the three horizontal rows in this table will vary with the respective values of E. For if we deduct the sum of the values for any one row from the sum of those in column L, the value they have in common cancels out and the remainder is of the form $(IX/IX+IV/IV)-(IX/IV+IV/IX)$, that is, $2E_{ix.\,iv}$. The differences between the mean values for the rows is therefore called the '*Union Factor*', for it may be regarded as a measure of the relative success of the various kinds of reciprocal union. If one value of E were significantly lower than the others it would indicate that the reciprocal unions between the two varieties concerned were less successful than the others. The difference between the mean values for the two columns U_1 and U_2, here called the '*Consistency Factor*', reflects the tendency for the relative effect of rootstock and scion on tree weight to be the same in each pair of varieties and for the relative success of each type of union to be the same in each case. For example, if the scion tended to be dominant in the top row where M.IV is combined with M.XII, and the rootstock tended to be dominant in the other two rows, where M.IX is combined with M.XII or M.IV, the mean values for U_1 and U_2 would tend to be unlike; for the value for IV/IV would tend to occur twice in U_1, i.e. in the top and bottom rows, and the value for XII/XII twice in U_2, i.e. in the top and middle rows. Again if the value for the equivalence factor for the reciprocal unions between M.IV and M.XII were due to the IV/XII rather than to the XII/IV trees and the equivalence factor for the reciprocal unions between M.IX and M.XII were due to the XII/IX rather than to the IX/XII; this inconsistency in M.XII when used as a scion would result in the value of U_1 being less than that of U_2.

The values for the variance ratio (F) for each factor are shown in Tables VI and VII, which refer to the real values and to their logarithms respectively. The degrees of freedom associated with each factor are shown in the column headed 'd.f.' and those for appropriate value for 'error' at the base of the tables. The August 1937 and in the final harvest of residual trees were not harvested according to blocks or rows, all the remaining degrees of freedom, after subtracting the 8 for kinds of tree, are therefore allocated to 'error'

Table VI

Values of the Variance Ratio (F) for the Various Factors affecting Growth, obtained in Analyses of Variance on the Fresh and Dry Weights of the Nine Kinds of Tree

Source.	d.f.	Fresh or dry.	Aug. 1937.	Aug. 1938.	April 1939. Routine sample.	April 1939. Final air-dry sample.
Rootstock	2	F.W.	49·09***	70·90***	67·45***	182·99***
		D.W.	46·88***	70·16***	72·44***	—
Scion	2	F.W.	4·05*	2·70	12·96***	24·30***
		D.W.	4·19*	1·86	13·82***	—
Equivalence (L–U)	1	F.W.	2·77	3·53	7·12*	16·20***
		D.W.	2·34	4·32*	7·81**	—
Union	2	F.W.	2·14	1·95	1·81	2·85
		D.W.	1·92	2·45	1·67	—
Consistency (U_1–U_2)	1	F.W.	0·47	0·38	0·44	2·41
		D.W.	0·44	0·44	0·45	—
Degrees of freedom in error	Rootstock	45		9	9	171
	Scion and interactions			29	29	

Table VII

Values of the Variance Ratio (F) as in Table IX but obtained in Analyses of Variance on the Logarithms of the Fresh and Dry Weights

Source.	d.f.	Fresh or dry.	Aug. 1937.	Aug. 1938.	Apr. 1939. Routine sample.	Apr. 1939. Final air-dry sample.
Rootstock $P =$	2	F.W.	45·74***	22·90***	67·79***	176·5***
		D.W.	45·30***	22·92***	72·18***	
Scion	2	F.W.	4·92*	2·02	11·53***	19·50**
		D.W.	5·65**	1·18	11·09***	
Equivalence (L–U)	1	F.W.	0·72	0·87	1·83	2·71
		D.W.	0·63	1·44	2·12	
Union	2	F.W.	3·50*	1·17	0·37	0·55
		D.W.	3·76*	1·46	0·32	
Consistency	1	F.W.	0·38	0·20	0·35	1·93
		D.W.	0·35	0·19	0·40	
Degrees of freedom in error	Rootstock	45		10	10	171
	Scion and interactions			30	30	

which is the same for all factors. In 1938 and 1939 the 17 degrees between the 18 rows are made up of 2 between rootstock varieties, 5 between blocks, and 10 allocated to $Error_1$. The remaining 36 degrees, within rows, represent 2 between scion varieties, 4 for the interactions, and 30 for $Error_2$. In Table VI the 10 and 30 degrees were reduced to 9 and 29 by the loss of 1 degree for regression with the 'fertility gradient'.

Rootstock factor. The variance ratio is very highly significant in every case in both tables and is of the same order of magnitude whether the analyses have been carried out on the actual weights or on their logarithms. The only considerable divergence is in the values for 1938, where they are about 70 and 23 respectively. This was largely the outcome of the adjustment for drift, carried out in one case and not in the other; the unadjusted value was about 30 instead of 70.

Scion factor. The values of the variance for the ratio for scion effect were considerably lower than those for rootstock effect. In 1937 they were just significant, in 1939 very significant; in 1938 they failed to reach significance.

Equivalence factor. In Table VI the ratio is at about the $P = 0{\cdot}05$ level in 1938 and the $P = 0{\cdot}01$ level in 1939; in the final sample it is highly significant. In Table VII, however, the values never approach significance. Thus this factor was significant when based on the differences between the arithmetic means, but not when based on the logarithms of the ratios between their geometric means. The probable meaning of this is discussed more fully in a later section.

Union and consistency factor. In only one case did the variance associated with either of these factors approach significance; this was for union in August 1937 in Table VII. It is unlikely that this has any real importance as the value only just reached the $P = 0{\cdot}05$ level. When dealing with a large number of comparisons, we should expect some to reach that level by chance.

Values for fresh and dry weights. The values of the variance for fresh and dry weights were practically identical in each table. To avoid duplication, therefore, only the values for the fresh weights will be given in subsequent tables.

Relative Effects of Rootstock and of Scion

The mean fresh weights of trees on each rootstock, and the ratios between their geometric means, are given in the left-hand portion of Table VIII; the ratios between the values on two successive occasions, and the ratios between these ratios, are given in Table IX. The corresponding values for the trees grouped according to the variety used as scion are given in the right-hand portions of these tables.

The values for rootstock given here are the mean values for all trees on a given rootstock, irrespective of the variety used as scion, e.g. the value for —/IX is the mean of those for IX/IX, IV/IX, and XII/IX. It is of interest, therefore, to compare these values with the corresponding values in Tables I and II, which were concerned solely with trees with 'Like' unions, e.g. IX/IX.

The values follow the same general pattern as in Tables I and II, but the differences are almost always less than for 'Like' trees and the ratios nearer to unity, indicating that the weight of a composite tree was here largely governed by the rootstock variety, but the presence of a scion of another variety tended to make the trees a little more alike. Only one of the ratios between ratios in Table IX is significant.

Table VIII

Rootstock and Scion Effects Compared. Fresh Weights of Non-deciduous Trees on Each Rootstock and of those worked with Each Scion, during the First and Second Scion Years, and the Ratios between the Weights of Each Pair

	Rootstock effect				Scion effect		
Date	Aug. 1937	Aug. 1938	Apr. 1939		Aug. 1937	Aug. 1938	Apr. 1939
Trees per unit	18	6 groups of 3	6 groups of 3		18	6 groups of 3	6 groups of 3
At time of:	Scion maiden year	Scion second year	End of second year		Scion maiden year	Scion second year	End of second year
	Mean weights						
Rootstock	*g.*	*g.*	*g.*	Scion	*g.*	*g.*	*g.*
−/IX . .	93	{452	{500	IX/−	120†	{662	{824
−/IV . .	79	{629	{891	IV/−	{96	{711}	{877
−/XII . .	159	1,122	1,686	XII/−	{115†	830}	1,377
	Ratios						
−/IX:−/IV	1·17*	*0·75*	0·49*	IX/−:IV/−	1·28*	*0·92*	*0·97*
−/IV:−/XII	0·50***	*0·58*	0·54**	IV/−:XII/−	*0·88*	*0·87*	*0·65*
−/XII:−/IX	1·73***	2·33**	3·10***	XII/−:IX/−	*0·89*	*1·25*	1·57***

The mean weights for 1938 and 1939 have been adjusted by co-variance for the 'fertility gradient' from north to south of the plot.

Bracketed weights are not significantly different. Ratios in italics do not differ significantly from unity.

* $P = 0{\cdot}05$ ** $P = 0{\cdot}01$ *** $P = 0{\cdot}001$

† Not significantly different.

These indications are further borne out by the values for the scions given in the right-hand half of the tables. When grouped according to scion, the differences are less than when grouped according to rootstock, but some are nevertheless significant. In August 1937 trees worked with M.IV were significantly lighter than those worked with M.IX. In 1938 and 1939 they tended to be heavier but not significantly so: the ratios between their geometric means were practically unity. Trees worked with M.XII weighed about the same as those worked with M.IX in 1937, but had become very significantly heavier than those with either of the other two varieties as scion by April 1939, mainly on account of a greater relative increase in weight between August 1938 and April 1939: it seems that trees with this scion continued growth

Table IX

Rootstock and Scion Effects. The Ratio between the Weight on One Occasion and that on Another for Trees on the Same Rootstock and for those worked with the Same Scion, and the Ratios between these Ratios

	Rootstock effect				*Scion effect*		
Occasions	Aug. 1937	Aug. 1938	Aug. 1939		Aug. 1937	Aug. 1938	Apr. 1939
Compared	Apr. 1937 (after).	Aug. 1937.	Aug. 1938.		Apr. 1937 (after).	Aug. 1937.	Aug. 1938.
			Ratios				
−/IX . .	2·25***	5·00***	*1·11*	IX/− . .	2·34***	5·15***	1·25*
−/IV . .	2·22***	7·83***	*1·40*	IV/− . .	1·84***	7·15***	*1·18*
−/XII . .	1·79***	6·74***	*1·48*	XII/− . .	2·09***	7·19***	1·57***
			Ratios between ratios		†		
−/IX:−/IV .	*1·01*	0·64*	*0·79*	IX/−:IV/−	1·28*	0·72**	*1·06*
−/IV:−/XII .	*1·24*	*1·16*	*0·95*	IV/−:XII/−	*0·88*	*0·99*	*0·75*
−/XII:−/IX .	*0·79*	*1·35*	*1·33*	XII/−:IX/−	*0·89*	1·40*	*1·26*

† As the correct value to use for April 1937 is the general mean, these *ratios between ratios* for (August 1937: August 1938) are the same as the *ratios* for (August 1937) in Table VIII.

Ratios in italics do not differ significantly from unity.

* $P = 0{\cdot}05$ ** $P = 0{\cdot}01$ *** $P = 0{\cdot}001$

Table X

The Equivalent Factor. Arithmetic and Geometric Mean Weights for Trees with Like and with Unlike Unions, and the Ratios of Unlike and Like on Each Basis

	Aug. 1937.	Aug. 1938.	Apr. 1939 Routine sample.	Apr. 1939 Final air-dry sample.
Arithmetic means	g.	g.	g.	g.
Like	119	815	1,202	1,042
Unlike	106	694	938	832
Significant difference . . .	15	99	179	134
Geometric means*				
Like	106	696	939	778
Unlike	100	636	830	706
Ratio U/L				
Based on A.M.s . . .	90	85	78	80
Based on G.M.s . . .	95	91	88	91

* Significant ratios for Geometric Means are not given as they never approached significance.

later into the season than trees with the other two scions. This is brought out also by the appropriate values in Table IX; these show that XII/— trees increased 1·57-fold—over 50 per cent.—during this period, and the IX/— and IV/— trees only some 25 and 18 per cent. respectively. The ratios between ratios for this period, however, did not differ significantly from unity. The higher levels of significance for ratios between ratios, for the period August 1938/August 1937, was due largely to greater precision, resulting from there being 30 degrees of freedom associated with error for scion effect, and only 10 degrees for rootstock effect. Although the values for mean weights shown in Table VIII provide a valid picture of the relative sizes of the trees on the three rootstocks and those worked with the three scions, they can be rather misleading if we are considering the active effect of rootstock, and of scion, during the period 1937 to 1939. For the weights of the trees on the three rootstocks must be compared with those of the three rootstock varieties at the end of the rootstock year, that is, with those in column April 1937 (After) in Table I, but the weights of the trees worked with the three scions must be compared with the general mean for that date, for the same number of trees of each rootstock were worked with each scion. Only part of the differences between the weights of trees on the three rootstocks can be accounted for in this way. The difference in relative weight of trees on M.XII and on M.IX increased during the period 1937 to 1939, and trees of M.IV started lighter than those on M.IX and became significantly heavier.

The relative growth rates for trees on the three rootstocks and those with the three scions are of interest in this connexion. These values, worked out from those in the upper part of Table IX, were 126, 160, and 144 for the three rootstocks and 136, 137, and 158 for the three scions.

The Equivalence Factor. Relative Weights of Trees with Like and Unlike Unions

One of the main purposes of this experiment was to examine the relation between the size of the composite tree and those of simple trees of the component varieties. The values of Table X provide evidence on this point. The arithmetic means of the weights for trees with Like and with Unlike unions are given in the upper part of the table, together with the differences required for the $P = 0{\cdot}05$ level; as already shown by the analysis of variance (Table VI), this difference was significant in 1938 and 1939.

The differences between the mean values of their logarithms, however, were not significant (Table VII), and the values for the geometric means, found by back-transformation of these logarithms, are therefore of interest and are given in the middle part of Table X; clearly the differences are here much smaller. The ratios of Unlike to Like are shown at the base of the table; those based on the geometric means are much nearer unity than those based on the arithmetic means. Although the ratio between the G.M.s never differed significantly from unity, it was consistently lower and this difference

was probably real. The lower values found where G.M.s are used suggests that the difference, in so far as it exists, tends to be proportional to the summed mean weight of the trees concerned.

Discussion

The experiment was planned to provide information on three problems, two of which are dealt with in this paper.

The first is the relation between the growth rates of composite trees and those of trees of the component varieties. This has been examined by comparing the summed weights of trees with reciprocal grafts (Unlike unions) between two varieties, e.g. IX/XII and XII/IX, with those of the trees composed entirely of one or other variety (Like unions), e.g. IX/IX and XII/XII. The answer depends in part upon whether this 'equivalence factor' is based on the differences between the arithmetic means or on the ratio between the geometric means. On the first basis the difference between trees with Like and those with Unlike unions became significant during the second year from the scion bud; the Unlike trees weighed about 20 per cent. less than the Like by the end of that season. On the second basis, however, the difference was only about 10 per cent. and was never significant. The second, or geometric, basis is probably to be preferred; for increments in size in young trees are likely to be related to the size of the tree, and the *relative* growth rate ($\log_e W_2/W_1$), rather than the *actual* growth rate ($W_2 - W_1$), seems the proper measure for the effect of factors such as these.

Although the ratios were never significantly different from unity on any one occasion, and were never very far from it, they were consistently lower, and this probably represented a real difference between trees with Like and Unlike unions. This does not necessarily mean that their growth rates were not, in the mean, the resultant of those of the constituent varieties or could not have been calculated from a knowledge of the latter. It is possible that, in general, the partnership between components in an Unlike union may not be quite as efficient as that in a Like union. Varieties differ in their natural times for starting growth in spring and ending it in autumn and in their periods of maximum growth; moreover their cambiums may start activity at different dates. The mutual adjustments required to make the composite tree an efficient mechanism may result in a reduction in growth. It should be noted, however, that the equivalence factors for the three pairs of varieties—M.IX and M.IV, M.IV and M.XII, and M.XII and M.IX—were not significantly different. Even if the growth rate of a composite tree was the resultant of those of the component varieties, and approached that of the one or of the other, according to which formed the greater part of the tree, it does not necessarily follow that complete equivalence would result.

The second problem on which further information was sought was the relative influence of rootstock and scion in composite trees. Here the answer has been definite and in accordance with all past findings at East Malling.

The rootstock had far more influence than the scion in determining tree weight. Scion variety, however, had some influence, as already demonstrated at East Malling some thirty years ago (Hatton, Grubb, and Amos, 1924). Although part of the difference in the relative weights of trees on the three varieties of rootstock, at the end of the second year of scion growth, was a reflection of differences already established before the scion bud grew out, considerable changes in relative weight took place during this period. In particular, trees on M.IX grew at a considerably slower rate than the others. At the start those on M.IX were heavier than those on M.IV and more than half as heavy as those on M.XII; at the end they were lighter than those on M.IV and less than a third the weight of those on M.XII. Attempts were made to determine more exactly the periods during which the various rootstocks exerted differential influences on tree growth, by comparing the relative growth rates during successive periods and the relative weights on successive occasions. These attempts were not altogether successful owing to the variability of the material; the experiment was not designed to yield data suited to critical examination of fine distinctions such as differences between differences and ratios between ratios.

Sufficient information was obtained, however, to justify this method of attack and to suggest that the method might be very fruitful with data from experiments planned for this purpose. It is largely on this account that a technique is being developed for growing trees with their roots in a mist of cultural solution (Vyvyan and Trowell, 1953); it seems possible that variability may be reduced by ensuring uniformity in nutrient conditions in this way.

This experiment was concerned only with young trees during the 3 years after planting; the 'rootstock year' and the first 2 years of scion growth. During the first of these years the trees were composed entirely of the rootstock variety, during the maiden year of scion the rootstock was still the greater part of the tree, but during the second scion year the scion became the larger part. It would be of interest to know how far differences in size of older trees on various rootstocks are the result of differences established during these early years and how far they are brought about by subsequent differences in relative rates of growth. Though the scion will form the larger part of such trees, the rootstock will still be a considerable portion, probably at least a third under any system of working; and may, therefore, have considerable influence. The growth responses that result from scion-rooting and from inarching with different rootstocks seem to point in this direction.

Summary

1. One-year-old rooted shoots from stool-beds of the apple rootstock varieties M.IX, M.IV, and M.XII were planted in 1936 and some of each were budded the same year with the same three varieties to form trees of the nine possible combinations of the three varieties.

2. The weights of sampled trees of each kind were determined at intervals during 3 years from planting, that is, two from the outgrowth of the scion bud.

3. During the first or 'rootstock' year—before the scion bud grew out, trees of the three varieties grew at about the same relative rate: during the next 2 years the trees of IX/IX grew relatively slower than those of IV/IV and XII/XII.

4. The ratio of the mean weight of trees with reciprocal Unlike unions, e.g. IX/IV and IV/IX to that of Like union trees of the component varieties, e.g. IX/IX and IV/IV, was consistently about 0·9, but the deviation from unity was never significant. This indicates that the relative growth rate of a composite tree is mainly the resultant of the growth rates of the component varieties.

5. Rootstock was found to have a greater influence than scion variety on tree size, and composite trees, e.g. IX/IV, usually resembled those of the rootstock variety, e.g. IV/IV, more than the scion variety, e.g. IX/IX. Scion variety had some effect on tree size when M.XII was the scion.

6. Part of the difference in size of trees on the three rootstocks, at the end of the second year of scion growth, represented differences established before the scion bud grew out, but much was due to differences in relative growth rates during the period.

7. Methods for determining the periods of differential growth rates by examining the ratios between the relative growth rates are described and discussed.

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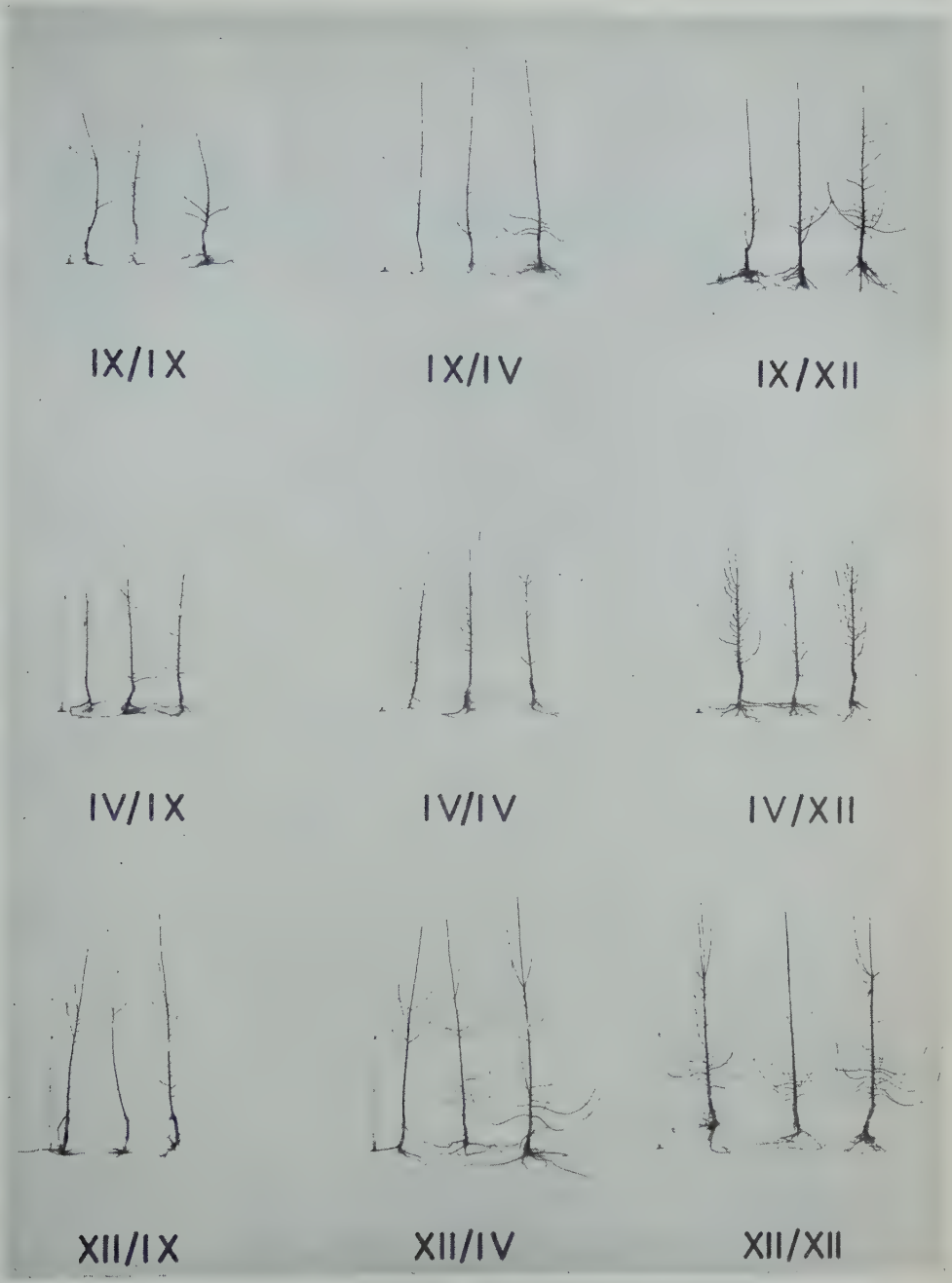

Typical trees of each scion-rootstock combination, two years from the scion bud. The corresponding values for Mean Fresh Weights are presented in Table IV

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A Review of the Genus *Sporocarpon* Williamson

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With Plate XV

ABSTRACT

The genus has been reviewed and reclassified after examination of Williamson's slides in conjunction with the study of more recently discovered material. *Sporocarpon pachyderma* (Williamson) is shown to be a fungal structure, its appearance in the Carboniferous being the first record of a Palaeozoic septate fungus. The remaining species have been divided into two form genera because of major differences in structure.

INTRODUCTION

THE genus *Sporocarpon* was established without precise definition by W. C. Williamson (1878) on the basis of certain objects which he says '. . . appear to be conceptacles of some kind that have formed in the midst of parenchymatous tissue, but from which they have shown a remarkable tendency to become detached by a somewhat definite yet irregular contour. . . . I propose grouping these and some allied objects into the provisional genus *Sporocarpon*.' In 1880 he introduced new species, which were not formed in the midst of parenchymatous tissue but consisted of 'conceptacles' lying freely in the matrix. He then amplified the description of the genus by saying: 'one common feature characterises the whole of the objects which I have included in the provisional genus *Sporocarpon*, viz: they exhibit no trace of having possessed any peduncular appendage wherewith to be attached to their parent plant.' He gives no other criteria for the genus, and these given are so unspecific that many megaspores and other objects without 'peduncular appendages' could be included in it.

He described *S. cellulosum*, the first discovered and presumably 'type' species, in 1878, and he subsequently described six other species: *S. elegans*, *S. compactum*, and *S. tabulatum* (1878); *S. pachyderma*, *S. asteroides*, and *S. ornatum* (1880). In 1883 he described a section which he identified as *S. ornatum* in discussion but which he labelled as *S. annomalum* in the descriptions of the illustrations in the same article. *S. furcatum* was named by J. W. Dawson in 1888 and described in detail by Kidston and Lang (1924). The characters of this organism do not coincide with those of the genus as described by Williamson, in particular in that the structures are not detached from other tissues, and it was transferred to the genus *Foerstia* by White and Stadnichenko (1923).

The scattered and incomplete descriptions of some of these species, and apparent typographical or other errors in some cases, caused difficulty during

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a recent detailed study. Williamson's accounts and slides have therefore been collated and re-examined in conjunction with a study of more recently discovered material. There are many significant differences between the species which make it difficult to accept his grouping of them into a single genus, and his diagnoses do not conform with the requirements of the International Rules for Botanical Nomenclature. The following reclassification is therefore proposed:

Mycocarpon nov. gen. One species:
Mycocarpon pachyderma (Williamson) nov. comb. (Type.)
syn. *Sporocarpon pachyderma* Williamson.

Sporocarpon Williamson (amend.). Two species:
Sporocarpon cellulosum Williamson (amend.). (Type.)
Sporocarpon asteroides Williamson (amend.).

Dubiocarpon nov. gen. Three species:
Dubiocarpon elegans (Williamson) nov. comb. (Type.)
syn. *Sporocarpon elegans* Williamson.
Dubiocarpon compactum (Williamson) nov. comb.
syn. *Sporocarpon compactum* Williamson.
Dubiocarpon tabulatum (Williamson) nov. comb.
syn. *Sporocarpon tabulatum* Williamson.

Fossiles Incertae Sedis:
Sporocarpon annomalum Williamson.
syn. *Sporocarpon ornatum* Williamson.

These proposals are discussed below.

Mycocarpon nov. gen.

Fossil fungal structures containing spherical cavities surrounded by a wall made up of one or more layers of septate hyphae. Type species: *Mycocarpon pachyderma* (Williamson) nov. comb.

Mycocarpon pachyderma (Williamson) nov. comb. Spherical fossil fungal structures approximately 275μ to 600μ in diameter, each typically containing a single spherical cavity approximately 175μ to 260μ in diameter within a wall made up of septate hyphae. In some cases two cavities have a common wall in some part of their circumference. The wall is made up of an interwoven mass of branching hyphae which are approximately 5μ to 20μ in diameter, mostly 8μ to 15μ. Large oval cells approximately 40μ to 70μ by 25μ to 110μ in diameter may be present in the outer layers of the wall. The cavity may contain an apparently structureless membrane approximately 125μ to 225μ in diameter. A group of rounded cells with rough walls, 3μ to 9μ in diameter, or a group of thin-walled cells 20μ to 50μ in diameter, may lie irregularly within the membrane. Type specimen: Slide 1503, Williamson Collection, British Museum (Natural History). (Pl. XV, Fig. 1.)

This revised description is based upon a re-examination of Williamson's

slides together with an examination of 23 specimens from the Kidston Collection, 17 specimens from the Departmental Collection in the University of Glasgow, and 50 specimens in a series of serial sections cut from a coal ball from the Werister Colliery, Liége, Belgium. The measurements of the specimens are given in Table I.

The structure of the wall of the material collected at the Werister Colliery is less well preserved than in the other sections, which are all from the Halifax Hard Bed, but the specimens are all of similar habit. Their spherical structure can be confirmed by examination of the frequently occurring tangential sections, and of the serial peel sections. Most of the sections lie freely in the matrix, but some lie in close contact with each other (Pl. xv, Fig. 2). In these

TABLE I

Measurements of Mycocarpon pachyderma in μ

	Williamson Collection.	Kidston Collection.	Glasgow University collections: Departmental Collection.	Glasgow University collections: Werister Colliery.
Overall diameter . .	325–600	275–500	325–475	300–375
Diameter of cavity . .	185–260	175–250	180–275	200–230
Diameter of internal membrane	125–150	100–225	—	150–175
Diameter of cells in cavity.	3·6–8·5	None	—	20–50

cases the cells of the wall may form a continuous growth around the two cavities as reported by Williamson, but there is no indication of any substantial 'stroma' in which the objects could have been formed. The wall of the structure has invaginated into the cavity in some cases, the resulting appearance being very like that of a collapsed cleistocarp (Pl. XV, Fig. 3). The wall is usually 4 to 8 cells thick and in most specimens it resembles that of the type specimen in that the hyphae end irregularly on the surface without any definite limiting layer. In one specimen on Kidston Collection slide 965 (Pl. XV, Fig. 4) the wall is surrounded by a ring of large cells measuring 40 μ to 70 $\mu \times 25\,\mu$ to 110 μ. Typical hyphae run between these cells and out into the surrounding matrix. Traces of similar cells can be seen in other sections, and smaller cells of a similar type are frequently found embedded in the walls. The outer surface of the wall as seen in a tangential section appears as a closely interwoven mass of cells precisely resembling those of the surface of a fungal cleistocarp. There is no sign of any opening to the exterior in any section, the wall being similar in structure in all planes. An inner membrane 0·5 μ to 1·0 μ thick is present in the cavity of the great majority of specimens, and in one specimen two concentric membranes are present. The cells described as 'granular bodies' by Williamson are present in the type specimen only, and the larger rounded cells have been seen only in the sections from the Werister Colliery. In many other sections ill-defined dark objects and

areas can be seen within the cavity, but details of their structure cannot be distinguished with certainty.

Williamson commented on the similarity of the 'branching tubules' of the wall to fungal hyphae, although he stated that he knew of no fungal structure which resembles this material in its entirety. The wall structure is, however, precisely similar to that of a fungal cleistocarp both in transverse section and in surface view, and both the occurrence of occasional specimens in pairs with a common continuous wall, and the type of invagination of collapsed specimens is very similar to the ascomycete habit. The nature of the cells seen on the Kidston slide 965 is uncertain. Similar cells are formed irregularly in other specimens, but they are less prominent and often smaller. If they are an integral part of the wall there may be justification for classifying the specimens in which they occur as a separate species or variety. They may, however, be parts of a host or other tissue in which the organism was growing. There are similar cells in the surrounding matrix, and the hyphae present between these large cells are similar to fungal hyphae growing within a host. The position of the membrane within the cavity corresponds exactly with the position of the group of ascogenous hyphae borne on the inner layer of enlarged wall cells which is common in the Ascomycetes. No clearly defined structures can be seen to be connected with this membrane, and although the dark objects to be seen in many cavities occasionally have some resemblance to hyphae, they are thought to be too indistinctly defined to be considered as substantial evidence for identification. Hutchinson and Walton (1953) thought that the appearance of the sections which they first examined justified a presumption that they were of an ascomycete, though they commented on the lack of definite evidence of an ascus. The failure to find either asci or definite ascogenous hyphae in the large number of specimens subsequently examined reduces the support for this presumption, however, and it is now thought more satisfactory merely to point out the clear similarities which are present.

Sporocarpon (Williamson) amend.

Spherical bodies containing a spherical cavity and showing no sign of vascular tissue. The tissue surrounding the cavity consists of parenchymatous cells. It has no definite external boundary, but exhibits an irregular surface of broken cells. The structures show no other sign of attachment to a parent plant. Type species: *Sporocarpon cellulosum* (Williamson) amend.

Sporocarpon cellulosum (Williamson) amend.

Spherical structures approximately 350μ to 500μ in diameter, each containing a single spherical cavity approximately 200μ to 400μ in diameter. The wall is made up of radial or fan-shaped rows of parenchymatous cells which are oblong in cross-section and whose radial walls are thicker than their tangential ones. The outer cells of the wall are broken, appearing as if they have been torn away from some enveloping tissue which is no longer present.

An irregularly arranged mass of rounded cells 25μ to 40μ in diameter may be present within the cavity, and each of these may contain two to four rounded endogenous cells 6μ to 10μ in diameter. Type specimen: Slide 1515, Williamson Collection, British Museum (Nat. Hist.).

This description is based upon a re-examination of Williamson's slides, as no other material has been found. Detailed measurements of the specimens are given in Table II.

His statement that the diameter of the first described and presumably type specimen is '0·0012 in.' is apparently a misprint for '0·012 in.', the actual maximum diameter found on re-examination being 350μ (0·014 in.). The mass of cells which lie within the cavity of the specimen on slide 1519 were

TABLE II

Measurements of Specimens of S. cellulosum *in* μ

Williamson's slide No.	1515	1517	1519	1521
Maximum overall diameter	350	350	400	500
Maximum diameter of cavity	300	250	300	400
Diameter of cells in cavity	25–30	None	25–40	None

described by him as 'parenchymatous'. They do not, however, form a continuous tissue, being mostly rounded and free from each other, and they are parenchymatous only in that they have unthickened and apparently unspecialized cell-walls. A spherical membrane is also present in the cavity of this specimen, which encloses a cavity 125μ in diameter. The above cells lie entirely outside this membrane, near to a gap in the wall of the structure.

The arrangement and structure of the cells of the wall is characteristic, the relative thickness of the radial cell walls compared with the tangential ones being reminiscent of the structure of a dehiscent sporangium of a higher plant. The tendency which Williamson describes for these cells to be arranged in 'fan-shaped' or radiating columns in three areas is only slightly evident, the cells being in similar columns in most parts of the wall. This appearance might also be interpreted as being due to the presence of well-developed tangential bands of thickening in a single layer of radially arranged cells. Careful study has produced no definite evidence to confirm this possibility or to disprove Williamson's interpretation. The absence of the outer layers of the structures make it uncertain whether they are actually parts of a higher plant, but since he clearly states that he based his genus on the characters of this species 'and certain other allied objects', it is thought that it must be taken as the type.

Sporocarpon asteroides (Williamson) amend.

Structures containing a spherical cavity approximately 200μ to 375μ in diameter. The wall is made up of parenchymatous cells which are prolonged outwards into an irregular number of conical rays, the maximum overall diameter of the structure including the rays being approximately 450μ to

650μ. The outermost layer of cells of the wall are broken or incomplete. One or more membranes may be present in the cavity; these may appear as a mass of dark dots 0·1μ to 0·3μ in diameter or as an apparently undifferentiated film. An irregular number of isolated cells approximately 25μ in diameter may be present in the cavity. Type specimen: Slide 1508, Williamson Collection, British Museum (Nat. Hist.).

This description is based upon a re-examination of Williamson's slide 1508 and on an examination of eighteen specimens on slide 853 from the Kidston Collection. The wall structure is characteristic, and numerous tangential sections on slide 853 confirm the conical shape of the wall outgrowths. An object on this slide which appears to be a young or small specimen has a cavity 75μ in diameter, surrounded by a wall of one or two layers of parenchymatous cells which is prolonged outwards at several places into rays. Each ray consists of a single radial row of cells surrounded at the base by a single layer of irregularly arranged parenchymatous cells. The cells in the central row appear to have been formed by the division of one radially elongated cell.

Most of the outermost layer of cells of the large specimens are damaged. There is no evidence to show whether this damage is due to the organism having been torn away from enveloping tissue or to damage which has occurred during fossilization. The complex shape of the structure and the undistorted shape of the remaining cells suggest that it is unlikely that it has been torn out of enveloping tissue, and the outline is much more uniform than that of *S. cellulosum*.

Dubiocarpon nov. gen.

Spherical structures containing a spherical cavity and showing no sign of vascular tissue or of attachment to any parent plant. The wall of the structure is made up of a single layer of radially elongated cells, which may have an irregular number of smaller cells in the spaces between them. Type species: *Dubiocarpon elegans* (Williamson) nov. comb.

Dubiocarpon elegans (Williamson) nov. comb.

Spherical structures approximately 400μ to 700μ in diameter each containing a single spherical cavity approximately 280μ to 600μ in diameter. The wall is made up of a series of 'dumb-bell' shaped cells arranged perpendicularly to the surface of the organism. At the outer and inner surfaces of the wall the enlarged extremities of the cells are in unbroken contact, but the middle constricted portions are isolated from each other. The lozenge-shaped spaces between the constricted portions are filled with a thin-walled parenchyma. The outer extremities of some or all of the 'dumb-bell' shaped cells may be prolonged outwards into cylindrical hair-like structures which have simple, bifid, or trifid apices. The cavity may contain an apparently structureless membrane which may be empty, or may contain a small number of rounded cells 60μ to 88μ in diameter, or may contain a larger number of smaller cells 30μ to 50μ in diameter. Two or four endogenous cells 10μ to

20μ in diameter may be present within these cells. Type specimen: Slide 1524 Williamson Collection, British Museum (Nat. Hist.).

This description is based on a re-examination of Williamson's slides, together with an examination of eight specimens from the Kidston Collection. The measurements of the specimens are given in Table III. The measurements of the length of the appendages are not given in the table, as the only complete appendage seen still in contact with the cell-wall was 200μ long and many broken fragments of appendages were seen 150μ to 300μ long.

In one slide from the Kidston Collection a mass of branched septate cells is present within the cavity membranes. These cells are 1·5μ to 2·5μ in diameter and very closely resemble fungal hyphae. In one section they are

TABLE III

Measurements of Sporocarpon Dubiocarbon elegans *in* μ

	Williamson Collection.	Kidston Collection.
Overall diameter	500–700	400–625
Diameter of cavity	400–600	280–525
Diameter of wall appendages	25–30	20–30
Diameter of internal membrane	250–400	190–350
Diameter of small cells in cavity	30–50	18–30
Diameter of large cells in cavity	60–85	Not present

particularly clear (Pl. XV, Fig. 5); in other sections they appear as a dense mass surrounding the large rounded cells already described. In the type specimen the interstices between the cells within the cavity membrane are filled with material which has an indistinct similarity to the dense mass of the above cells, and very clear hypha-like cells have been seen in a structure on this slide which resembles a damaged megaspore. In this case they are growing inwards in a 'bouquet' from a single point on the cavity wall. These cells in most specimens on the slide from which Pl. XV, Fig. 5, was copied are entirely within the cavity membrane and show no contact with the wall, but in one case they pass through the membrane where it is in contact with the wall. They cannot be traced within the wall cells, though these show signs of disorganization at the point of contact (Pl. XV, Fig. 6). The 'bouquet' arrangement of some of these hyphae is similar to the arrangement of ascogenous hyphae in some perithecia, but this appearance could also be due to the invasion of an organism from a single point of penetration.

Dubiocarpon compactum (Williamson) nov. comb.

Spherical structures approximately 325μ to 450μ in diameter, each containing a single spherical cavity approximately 230μ to 300μ in diameter. The wall is made up of a single layer of oblong club-shaped cells, the inner ends of which are flattened and in close contact so as to bound a very regular cavity. These cells continue in close contact throughout their length. Their

peripheral ends may be blunt, mamillate, or project as short hairs. The cavity may contain an apparently structureless membrane, and round or oval cells approximately 15μ to 30μ in diameter may be present either within the membrane or scattered irregularly throughout the cavity. Type specimen: Slide 1514, Williamson Collection, British Museum (Nat. Hist.).

This description is based on a re-examination of Williamson's slides, together with an examination of 15 other specimens from the Kidston Collection, 1 from the British Museum, and 6 from the departmental teaching collection at Glasgow University. The measurements of the undistorted specimens are given in Table IV.

TABLE IV

Measurements of Dubiocarpon compactum *in* μ

	Kidston Collection.	Williamson Collection.	Glasgow University Collection.
Overall diameter	325–425	425	325–450
Diameter of cavity	240–280	300	275–300
Size of wall cells	40–60 × 18–20	40–50 × 18–20	50–65 × 18–20
Diameter of internal membrane	175–200	160	250
Diameter of rounded cells in cavity	25–30	—	18–25
Maximum diameter of oval cells in cavity	16–25	—	—

The peripheral ends of the wall cells may be blunt or mamillate, or they may project to form hairs, but there is no sign of the typical 'dumb-bell' shaped cells which are found in *D. elegans*. A pale brown membrane is present in the cavity of many of the specimens, and in two sections there are two such membranes, the outer one lying very close to the inner surface of the wall and looking as if it had become detached from it. There are no other visible structures in the cavity of the majority of the sections. In three cases, however, freely lying cells are present. The section on slide 757 of the Kidston Collection shows three rounded cells, 25μ to 35μ in diameter, apparently embedded in a mass of otherwise amorphous material which fills the cavity, and that on slide 55 of the departmental teaching collection contains four similar cells 15μ to 25μ in diameter. The section on slide 965 of the Kidston Collection contains a number of rounded or oval cells 16μ to 25 μ in diameter scattered iregularly throughout the cavity both inside and outside the membrane, and in two areas apparently embedded between the wall cells. No similar cells have been seen in the matrix outside the section. Two large thin-walled cells 30 μ in diameter are also faintly visible within the membrane. The section on slide 16 from the Departmental Teaching Collection contains endogenous cells 13 μ to 15μ in diameter within the wall cells. The larger rounded cells in all these cases are similar to those found in other species of *Sporocarpon* (Williamson), but no other cells have been seen which resemble any of the smaller ones.

Dubiocarpon tabulatum (Williamson) nov. comb.

Spherical or oval structures approximately 270μ in diameter containing spherical or oval cavities 130μ in diameter. The wall is made up of a single layer of club-shaped cells arranged perpendicularly to the surface of the organism, the inner ends of which are flattened and in close contact so as to bound a very regular cavity. These cells are approximately 55μ to 75μ in length, approximately 4μ to 5μ in diameter at their inner ends, and approximately 8μ to 13μ in diameter at their peripheral ends. No structures have been seen in the cavity. Type specimen: Slide 1509, Williamson Collection, British Museum (Nat. Hist.).

This description is based upon a re-examination of Williamson's slide, which is the only specimen so far recorded, and it agrees generally with his original account. The wall cells are, however, smooth club-shaped structures, the 'flattened faces' which he describes being apparently his misinterpretation of the appearance of the shadows of underlying cells on one in correct focus.

The walls of *D. elegans*, *D. compactum*, and *D. tabulatum* resemble each other in that they are formed mostly from a single layer of regularly arranged radially elongated cells. These similarities are noted by Williamson, but the forms which he describes as 'intermediate' between *D. elegans* and *D. compactum* show gradations only in the shape of the cell apices and in the degree of elongation to form hairs. There is no indication in his slides, or in any of the others which have been examined, of any intermediate stages in the development of the characteristic 'dumb-bell'-shaped cells of *D. elegans*. Many of the sections of the two species are similar in size and their wall cells and the contents of the cavities appear to be in similar stages of maturity. It is therefore thought that his tentative merging of the two species is not justified.

Fossiles Incertae Sedis

Sporocarpon ornatum Williamson cannot be included in any of the above genera on account of its characteristic wall structure and the lack of evidence that it is spherical. It is noted that in his description of the type specimen Williamson stated that he was '. . . unable to determine whether, in its perfect condition, it is a cylindrical or a spherical body'. It is not clear on what evidence he subsequently changed his opinion and referred to them as 'spherical' bodies, as there is no indication from these specimens of their shape at right angles to the plane of the section and he does not report on the examination of other material. The specimens are not unlike sections of a vascular stem from which the central tissues have been removed, and it is therefore thought advisable to record them as Fossiles Incertae Sedis.

Discussion

There is little in common between the species reclassified above other than their small size, and in both *Sporocarpon* and *Dubiocarpon* the arbitrary character of the wall structure is chosen for convenience in establishing what are

thought to be 'form genera' only. There are, however, certain similar objects which are found irregularly in the cavities of most of the specimens. These are:

1. A 'membrane' which has already been discussed in *M. pachyderma*. In transverse section it is typically a brown ring of material about 0·5 μ to 1·0 μ thick; no details of its structure have been distinguished except in the type specimen of *S. asteroides*. The very regular occurrence suggests that it is a normal constituent part of the structures. A layer of apparently resinous material is common on the inner surface of the cellular wall. This membrane may be a similar layer which was deposited on the surface of a layer of cells which has since disintegrated or it may be a structure formed freely in the cavity. It usually appears to be quite smooth and even in outline, and not as if it is a remnant of disorganized cells. When more than one membrane is present the outer one may appear as if it had been pulled away from the inner surface of the cellular wall.

2. Irregularly arranged rounded cells up to 30 μ in diameter lying freely within the membrane in the cavity. These vary in number and size, but in most cases are similar in appearance. Their frequent occurrence in most of the species may indicate an overall relationship which is not demonstrated by other characters, or the presence of a common endophyte. It is noted that similar cells are very common in specimens of the genus *Traquaria*, which are frequently found on the same sections. Some of these cells in the specimens under discussion resemble freely arranged endosperm cells, while the formation of endogenous cells within them has some similarity to tetraspore formation.

3. Various sorts of smaller cells with more dense contents which have been seen very irregularly in *Mycocarpon pachyderma* and in *Dubiocarpon compactum*. They have been seen in one specimen only of each species; they are irregularly distributed and may be contaminants.

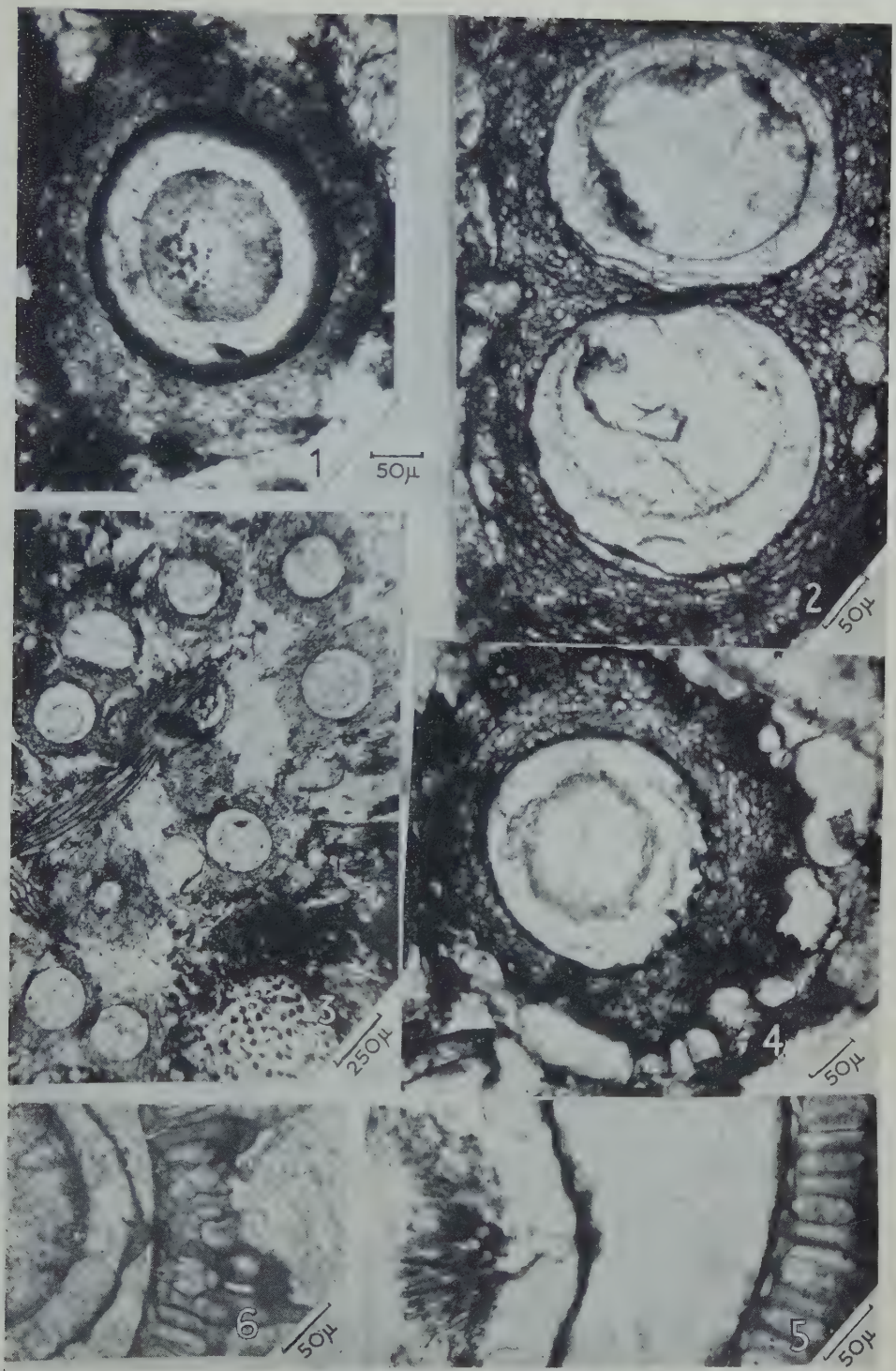
It is thought unlikely that the presence of these common cavity contents is a sign of close relationship between the diverse objects which have been examined. *Mycocarpon pachyderma* is thought to be a new species of Palaeozoic fungus, but while it is thought that the characters of wall structure and relative 'completeness' of the limiting layers justify the separation of the remaining species into two genera, their nature remains uncertain.

Summary

1. Williamson's description of the genus have been collated and extended after a study of his slides and of more recently discovered material.

2. *Sporocarpon pachyderma* Williamson has been found to be a septate fungal structure showing resemblances to a cleistocarp or a pycnidium, and it has been transferred to a new genus *Mycocarpon*.

3. Five of the remaining species have been reclassified into two new form genera:



S. A. HUTCHINSON

(*a*) *Sporocarpon* (Williamson) amend., including species having walls made up of several layers of irregularly arranged parenchymatous cells, and which are not bounded by a definite limiting layer.

(*b*) *Dubiocarpon* nov. gen., including species having a wall made up of a single layer of radially arranged cells.

4. *Sporocarpon ornatum* Williamson is removed from the genus as there is no evidence that it is spherical and it shows some similarity to higher plant tissue.

Acknowledgements

The writer is most grateful to Professor John Walton for bringing this material to his notice, and for his constantly helpful suggestions, criticism, and advice throughout the study. He is also indebted to the Trustees of the British Museum (Natural History) for the loan of the relevant Williamson slides, to Professor Suzanne Leclercq for the loan of the Werister material, and to Mr. W. Anderson for technical assistance.

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EXPLANATION OF PLATE XV

Illustrating Dr. S. A. Hutchinson's paper on *Sporocarpon* Williamson. Figs. 1 to 4. *Mycocarpon pachyderma* (Williamson) nov. comb.

Fig. 1. Type specimen showing small cells in cavity. Williamson Collection, Slide 1503.

Fig. 2. Two specimens with a common wall separating the cavities, showing wall structure. Kidston Collection, Slide 1127.

Fig. 3. Group of specimens showing habit. Glasgow University Figured Collection, Slide 647.

Fig. 4. Specimen showing large cells in outer layers of wall. Kidston Collection, Slide 965.

Figs. 5 and 6. *Dubiocarpon elegans* (Williamson) nov. comb.

Fig. 5. Portion of specimen showing a group of hypha-like cells branching from a single area on the cavity membrane. Kidston Collection, Slide 143.

Fig. 6. Portion of a specimen showing cavity membrane and hypha-like cells in contact with an area of the wall which contains distorted cells. Kidston Collection, Slide 143.

The Architecture of the Stem Apex and the Origin and Development of the Axillary Buds in Seedlings of *Acer pseudoplatanus* L.

BY

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With Plates XVI–XVII and seven Figures in the Text

Abstract

The structure and changes in the size of the stem apex of seedling sycamores are described. There is no evidence for any regular increase in size of the apex from pastochrons 6 to 14 inclusive.

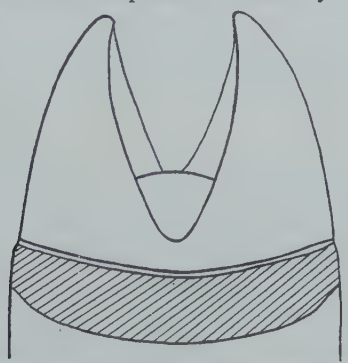
The formation of the collar by the bases of each pair of foliar primordia and its association with the formation of axillary buds has been studied.

The first pair of primordia on the axillary bud apex is always at right angles to the axil; the pair of primordia formed in the plane of the axil are always unequal. On extension growth these axillary buds give rise to lateral shoots which are anisophyllous. An explanation for the anisophylly is offered in terms of the spatial conditions obtaining in the developing axillary bud.

The vascularization of the apex is briefly described.

Introduction

THE leaves of the sycamore are arranged decussately. The leaf bases of each pair are extended laterally and fused to form a collar (or collet) surrounding the axis. The formation of this collar is a point of some significance in understanding the developmental anatomy of the plant.



Text-fig. 1. Diagram of stem apex of a sycamore embryo showing the first pair of leaf primordia. The shaded area is the cut surface of the collar region of the cotyledon which has been removed.

A sycamore embryo has a pair of long strap-shaped cotyledons. If these are separated and then pulled off, the apex, with a single pair of leaf primordia set at right angles to the cotyledons, can be seen. These two primordia are bluntly pointed, relatively stout structures. The collar formed by

the fusion of their lateral margins encircles the apex, only the tip of which is visible (Text-fig. 1). Thus with the exception of this first pair of leaves all the leaves formed by the seedling in its first season are produced during that time, and so mature in the same season as their initiation. During the first season of the seedlings lateral buds develop in the axils of all the leaves and also in the axils of the cotyledons.

Methods

Sycamore seedlings were grown over a period of 87 days in pots in a cool greenhouse. Samples of five plants were removed at approximately weekly intervals. The apices of these plants were fixed in chromic acid and have provided information on the structure of the apex and on the origin of the axillary buds.

The fixed apices were embedded in paraffin wax and sectioned either transversely or longitudinally. Sections were cut either at 6 μ or 10 μ and were stained in Heidenheim's haematoxylin and counter-stained in Orange-G in clove oil, to which a little absolute alcohol had been added.

Structure of the Apical Meristem

The apex has a single layered tunica over the corpus which is some seven or eight cells in depth (Pl. XVI). The flank meristem is generally not very sharply marked off from the rest of the corpus. It is most obvious as a zone in the earliest phase of the plastochron. At the base of the corpus is a cup-shaped zone in which the cell divisions are arranged in a cambial-like fashion. This is the region in which the basal cells of the corpus are developing into rib meristem, a zone of vacuolating and dividing cells, in which the division walls are orientated largely, but not entirely, in the transverse plane of the axis. Periclinal divisions do occur in this region; the tranverse expansion of the axis which is to be seen in this region below the apex is not entirely due to cell expansion.

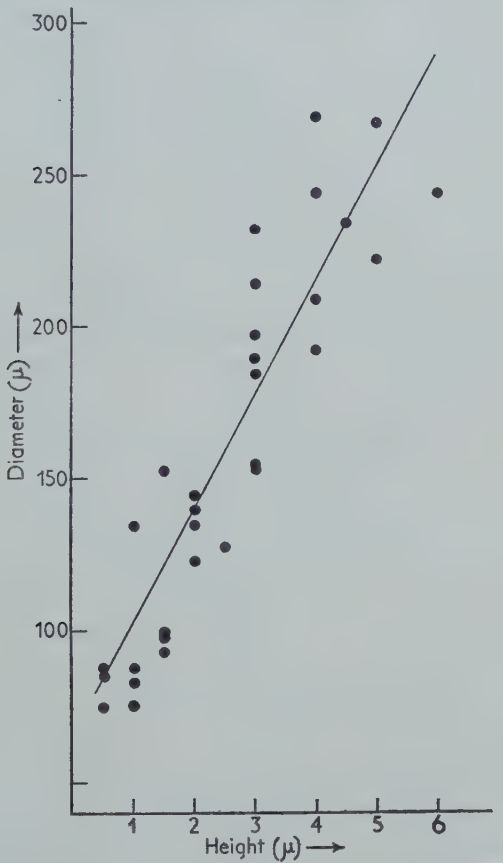
The presence of a cup-shaped zone with cambial-like divisions has been seen in the apices of other plants. Vaughan (1952) described such a zone in *Arabadopsis* and correctly interpreted it as adding to the rib meristem. But this zone is not, as Vaughan seems to suggest, strictly comparable to that described by Popham and Chan (1950) in *Chrysanthemum*. The zone described by these authors was found at certain stages of the plastochron situated *above* the level of insertion of the youngest foliar primordia, and while it did add to the zone of rib meristem it was also responsible, at least in part, for the growth in height of the apex following the initiation of foliar primordia.

The cambial-like zone of cells is not to be seen equally clearly in all apices of a plant. Its presence is probably not related either to the size of the apex (Popham and Chan, 1950) or to the phase of the plastochron (Vaughan, 1952). Most probably it is related to the intensity of growth in the apex and depends upon the balance between the rate at which the cells in the apex are

dividing and the rate at which the cells of the rib meristem are maturing to form the pith. Since each segment of stem formed elongates and matures in orderly sequence, there might be an accumulation of these rather shallow cells at the apical end of the rib meristem, if the apex is producing primordia at a rapid rate.

Changes in Shape and Size of the Apex during the Plastochron

During most of the plastochron the apex has the form of a dome. When a pair of primordia is formed the apex becomes almost flat, and of course its



Text-fig. 2. Diameter of the apex plotted against its height. The line is an arbitrary one inserted to show the general trend. Apex height and diameter are not strictly correlated (see text).

diameter is greatly reduced (Pl. XVI). The height of the apex has been measured above the level of insertion of the youngest pair of primordia. The height is least following the initiation of a pair of primordia, and it afterwards increases. The diameter of the apex changes in a similar fashion. In Text-fig. 2 apex diameter has been plotted against apex height. It can be seen from this figure that growth of the apex in height and in diameter is not strictly

correlated, although in general terms the lowest apices (which occur in the early phase of a plastochron) have the smallest diameters. This imperfect correlation probably results from the mode of growth of the apex. Increase in height is largely due to periclinal divisions in the corpus, while increase in diameter results mainly from anticlinal and oblique divisions in the flank meristem which result in the building up of the foliar buttresses.

There is little evidence of any regular increase in size of the apex from one plastochron to the next such as has been reported in certain monocotyledons (Abbe and Phinney, 1951; Ledin, 1954). The first plants sampled were developing their sixth pair of primordia and the last sampled their fourteenth pair. If apex diameter is plotted against plastochron number the differences appear to be related to the stage of the plastochron and not to the plastochron number. The same holds true for the height of the apex. Observation of the sections shows that when a pair of primordia is formed the apex becomes nearly flat (Pl. XVI), and measurements on these sections gave the minimum height obtained (5 μ). The greatest height found was 60 μ. This is a twelve-fold increase. If the extreme height of 60 μ is neglected, several apices were about 50 μ high, so we shall not be far out in estimating the increase in apical height *during* a plastochron as being of the order of ten times.

The minimum diameter recorded was 75 μ and the maximum 270 μ. If we regard the apex as circular in cross-section we can compare the area at the beginning and end of the plastochron:

$$\frac{\text{Maximum area}}{\text{Minimum area}} \quad \frac{135^2}{37{\cdot}5^2} = 13.$$

Thus the change in area during a plastochron appears to be of the order of thirteen times. While no evidence has been obtained of a regular change in the size of the apex, at any rate from plastochrons 6 to 14, it is not suggested that the apices cannot alter in size. It is clear that the apex of axillary buds, for example, does increase in size during development. It is a very small structure when its earliest primordia are formed, and later reaches a size similar to the main apex. It is noteworthy that the monocotyledons in which a regular increase in size of the apex from plastochron to plastochron has been described are all plants with very elongated apices, e.g. *Zea*; an extreme type found only very rarely, so far as I am aware, among dicotyledons. It may perhaps be related to the mode of growth and to the general absence of secondary thickening in these plants.

The depth of the non-vacuolated portion of the apical meristem (i.e. tunica plus corpus (*sensu stricta*)) remains more or less constant irrespective of the plastochron stage. Thus during the early stage of the plastochron the bulk of the non-vacuolated meristem lies below the level of the insertion of the primordia, while later a large part of it lies above the level of insertion of the primordia. If this region of the meristem remains more or less constant in depth, then the rate at which it increases in height during the plastochron must be about balanced by the rate at which the basal cells of the corpus

vacuolate and form part of the rib meristem. Vacuolation is not an instantaneous process and so the boundary between the non-vacuolated and the vacuolated regions is not precise. It is sufficiently so for measurements to be made under a low-power objective. The mean depth was 78 μ with extreme values of 65 μ and 100 μ.

Leaf Initiation and Formation of the Collar

Leaf initiation becomes obvious by divisions occurring in the tunica overlying the flank region. Subsequently or sometimes coincidently divisions

Text-fig. 3. Diagrams of transverse sections through successively lower levels of an apex. Regions of cell divisions involving both the apex and a primordium are indicated. The axillary bud (in the last five sections) is stippled.

occur in the underlying flank meristem. This activity results in the erection of a primordium, into the construction of which some of the corpus cells enter, and to the flattening of the apex (Pl. xvi).

Along the lateral margins of the bases of the primordia considerable cell divisions occur. This activity spreads around the side of the apex and so forms the collar. The cells formed are arranged in regular radial rows, and this zone extends down to the level of the next pair of primordia (Pl. xvi). It is in this zone that the axillary buds originate (see below). The distribution

of the cell divisions is such that the lateral margins of the bases of the primordia are 'fused' with the sides of the apex to a higher level than is the centre of the primordium base. Thus a small pocket is formed between the primordium base and the axis. This pocket is extended laterally in the plane at right angles to the primordium axil; it is deepest in the centre and becomes shallower towards the lateral margins. In the plane of the axil it is narrowest at its base and widest at the top.

At the stem tip the collars of several pairs of primordia overlap and only become separated later when internode elongation occurs. In Text-fig. 3 are shown diagrams of transverse sections through successively lower levels of an apex. Regions of cell divisions involving both the apex and the primordium base are indicated.

Origin of Axillary Buds

As stated above, the axillary bud meristem has its origin in the regular files of cells produced at the sides of the apex in connexion with the formation of the collar of the node, above the node at which the axillary bud is eventually situated. A small group of cells, the cells of the axillary bud meristem, become distinct as the surrounding cells vacuolate. The origin of the axillary bud meristem is always as a small group of cells. It is not possible to recognize a single cell stage in bud formation. The meristematic cells divide and this activity eventually involves some of the cells of the leaf base. The axillary bud meristem is 'left behind' in the primordium axil (i.e. in the base of the pocket formed by the collar), when the internode subsequently elongates; but in its origin, which is axial, it is associated with the leaves of the node above that at which it eventually stands. The cell divisions on the flanks of the apex which form the collar of the node provide the tissue in which the axillary buds of the two leaves of the node below form (Pl. XVII). In their regular arrangement these cell divisions resemble the shell-zones described by Schmidt (1924).

Sharman (1945) described a similar condition in the Gramineae, where the leaf base comes to encircle the axis by the rapid lateral spread of cell divisions from the margins of the young primordium; a condition essentially the same as the formation of the collar in sycamore, although here two opposite primordia are involved. Sharman (loc. cit.) considered that the development suggested that the bud should be associated with the leaf above the one in whose axil it will later appear to be situated. This view was supported by the way in which the subsequent behaviour was correlated with events in the leaf above rather than in the one below (Sharman, 1942). Sharman's account has been confirmed in *Zea* by Ledin in 1954.

The description given above for the sycamore indicates an axial origin for the axillary bud meristems, and this is in agreement with most writers on the subject. In some instances a foliar origin has been suggested. Majumdar and Datta (1946) described a completely foliar origin for the axillary bud meristems in *Heracleum sphondylium* L. and in *Leonurus sibiricus* L. The latter

plant is a member of the Labiatae, with opposite leaves. Most plants with opposite leaves show some development of a collar, but the authors do not describe this development in *Leonurus*, so it is not possible to judge whether conditions are similar to those in sycamore.

Axillary bud initiation is often said not to occur in the axils of primordia until they are some distance from the growing apex. Majumdar and Datta (loc. cit.) observed the first signs of bud initiation in *Heracleum sphondylium* in the axil of the fifth primordium from the apex, and in *Leonurus sibiricus* in the axils of the third pair of primordia from the apex. Gifford (1951) found in *Drimys winteri* that an axillary bud was perceptible in relation to the fourth primordium from the apex. Garrison (1949*a*), in her study of the origin and development of the axillary buds in *Syringa vulgaris*, states that 'when a leaf primordium occupies the position at the second or third node from the apex, a group of meristematic cells in its axil becomes delimited from the remaining cells of the apical meristem by the formation of a narrow zone of columnar cells'—a description which calls to mind the condition seen in the sycamore. In the apices of seedlings of this plant I have sometimes been able to recognize early stages of bud initiation in the axils of the second pair of primordia from the apex; but in other instances bud initiation is not obvious until a later stage. How early the axillary bud meristem becomes recognizably distinct probably depends upon the rate of development of the apex, since the axillary bud meristem becomes distinct by the vacuolation of the surrounding cells. If the development is rapid more primordia may be formed before vacuolation has occurred to delimit the meristem in the axil of a particular primordium.

I have never been able to detect any signs of axillary bud tissue in the axils of the youngest pair of primordia. This is to be expected if the interpretation of their origin given here is correct, since the tissue in which they will arise has not yet been formed.

Development of the Axillary Buds

Growth of the small patch of meristematic tissue isolated in the axil of the leaf leads to the formation of a small apex which stands in the pocket between the leaf base and the axis. The tissue at the base of this apex soon vacuolates so that the apex is mounted upon a short parenchymatous stalk, part of which is embedded in the tissue of the shoot at the level of attachment of the axillant leaf. The axillary bud apex, although at this stage much smaller than the terminal bud, has the same structure. Foliar primordia soon begin to form. In seedling sycamores the axillary buds forming in the axils of the primordia of the terminal bud in the first season develop leaf primordia which may expand in the second year. This is not always the case. Garrison (1949*b*) found that in the trees *Betula papyrifera* and *Euptela polyandra* primordia only develop on the axillary bud apex in the second season. Other plants are known, e.g. *Sambucus nigra*, in which bud primordia and leaves of the bud form during the same growing season. I do not know what happens in this respect in adult sycamores.

Orientation of the Primordia

The first pair of primordia formed on the axillary apex, which will, of course, develop as bud scales, are always orientated at right angles to the leaf axil. No exception has been seen in this work. An examination of the literature suggests that this orientation may be a general rule for plants with opposite leaves. Gifford (loc. cit.) found that the prophylls in *Drimys winteri* arose at right angles to the subtending leaf, and Garrison (loc. cit.) found the same thing in *Syringa vulgaris*. Although Majumdar and Datta made no reference to it, their drawings of transverse sections of the axillary bud of

Text-fig. 4. Longitudinal section of an axillary bud. The foliar primordium adjacent to the main axis is smaller than the opposite one. Apical meristem is stippled.

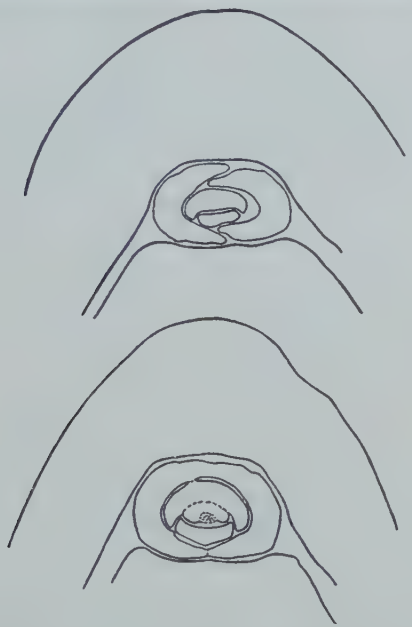
Leonurus figure the first pair of primordia orientated at right angles to the leaf axil. Esau (1953) shows a similar figure for the axillary bud of *Lonicera*.

In the sycamore the second pair of primordia to form on the axillary apex and all others subsequently formed which lie in the plane of the axil are unequal in size and rate of development, so the axillary apex becomes markedly asymmetrical. It is always the primordium adjacent to the main axis which is smaller and whose development is often retarded (Text-figs. 4 and 5). The development of the collar tissue of a pair of primordia lying at right angles to the leaf axil is usually delayed on the side adjacent to the main axis (Text-fig. 5). The general picture that is obtained is that cell division is less extensive and takes place more slowly on the side of the apex adjacent to the main axis.

Inequality of primordia of axillary buds has long been known (see Goebel, 1900) and leads when extension growth takes place to anisophylly of the lateral shoots (Goebel, 1900; Sinnott and Durham, 1923). The cause of this anisophylly clearly operates during the development of the axillary bud. Since the unequal primordia arise on an apex which is, at least at first, sunk in the

axil of a primordium which is still part of an apical bud, it seems unlikely that light can be the causal factor involved. The axillary bud apex stands more or less parallel to the main axis and so it is difficult to see how geotropism could be responsible at this stage for the unequal development of the primordia.

There remain at least two other possibilities: (1) unequal nutrition of the two sides of the apex, and (2) the spatial conditions under which the axillary bud forms. There do not appear to be any very obvious anatomical reasons why one-half of the apex should receive better supplies of nutrients than the



TEXT-FIG. 5. Two transverse sections through an axillary bud showing the orientation of the primordia and the inequality of those in the place of the leaf axil. In the lower figure the collar of the second pair of primordia is not yet formed on the axis side.

other. The vascular supply to the axillary bud is distinct from that to the axillant leaf (see below).

It seems to the writer that whatever explanation is offered for the inequality of the primordia in the axil of the leaf must also explain why the first pair of primordia are invariably formed at right angles to the plane of the axil.

The axillary bud develops in the pocket formed between the leaf base and the axis. Thus the apex will be, or will tend to be, compressed in the plane of the axil. This pressure on the sides of the apex could hinder cell division in these regions and lead to the first pair of primordia being formed at right angles to the plane of the axil. With the formation of the first pair of primordia and their attendant segment of stem, the axillary bud will increase a little in length and come to lie in a slightly wider portion of the pocket, since the axillant leaf is inserted at an angle to the axis. So there would be less

pressure on the side of the apex adjacent to the leaf, but pressure would be maintained on the apex on the axis side since the axis is vertical. The pressure induced by the axis would be increased by the marginal growth of the first pair of primordia, since the collar they form lies between the apex and the axis (Text-fig. 5). The space in which new primordia can form on the axis side of the apex is restricted. That the spatial relations obtaining in the leaf axil are not without effect upon the general form of the axillary buds is clear from Text-fig. 5. The elongation of the axillary bud in the plane at right angles to the leaf axil is obvious in all transverse sections of axillary buds examined during this investigation.

Evidence that the apical meristem of the axillary bud may be under some pressure has been seen occasionally in some buds where the cells of the tunica had bulging outer walls—a condition which could arise if the apex was compressed. Evidence supporting the view that it is the spatial relations which determine the inequality of the primordia is provided by the further development of the axillary buds. During the first season of extension growth the shoot is anisophyllous, but in the second season of extension growth *this degree of anisophylly is lost.* The leaves on this shoot have been formed by the bud, while the latter is morphologically the terminal bud of a lateral shoot and no longer confined to a leaf axil.

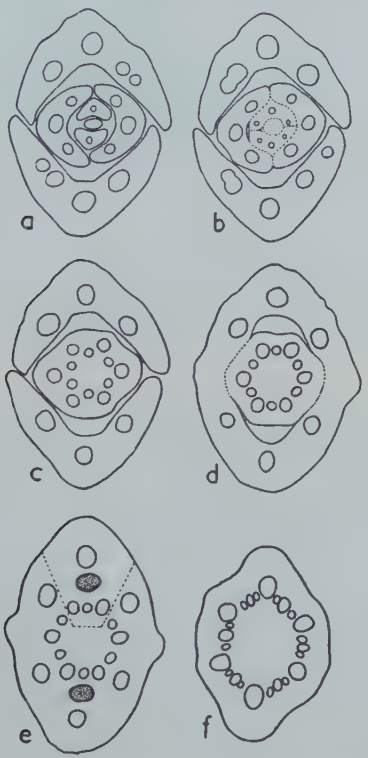
Vascularization of the Stem Tip

The vascular tissue of the very young seedling and the transition phenomena have been described by Holden and Bexon (1923), while Smith (1937) has described the nodal structure of mature shoots.

There are three procambial strands associated with each primordium, a median and two laterals. At the earliest stage at which it is possible to recognize a foliar primordium the procambial tissue is distinct at its base. In one or two instances the procambium has appeared to precede the appearance of the primordium. The procambial tissue is continuous below with the mature vascular tissue, and it develops acropetally in the primordium. In the primordium the median strand of procambium develops in advance of the two lateral strands.

The organization of the vascular tissue in the stem tip is most easily described by means of a series of diagrams representing the conditions at successively lower levels, starting at the apex and extending through three plastochrons. In Text-fig. 6*a* the youngest pair of primordia have only their median trace developed. The other primordia, each with their three vascular strands, are free from the apex. In Text-fig. 6*b* the first primordia are forming their collar and their lateral traces are developing. The second pair of primordia have also formed their collar and in this region are 'fused' to the axis. In Text-fig. 6*c* there is a ring of six vascular strands which are the traces from the youngest primordia. The traces from the second pair of primordia have not yet entered the vascular ring. They run obliquely through the collar

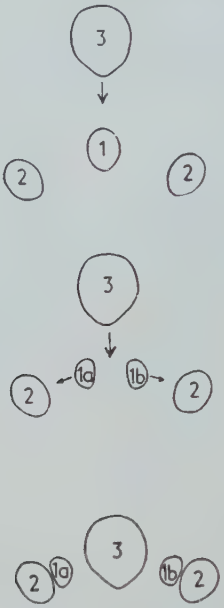
region before doing so. In Text-fig. 6*d* these traces have entered the ring and the third pair of primordia has formed its collar. In Text-fig. 6*e* the six traces from the third pair of primordia are running obliquely in the outer part of the axis. Before these traces enter the vascular ring leaf-gaps will be formed. The leaf traces from the third pair of primordia lie on the same radii as the traces from the first pair of primordia. The six traces from the first pair of primordia all behave in the same way. Each divides into two (leaving



TEXT-FIG. 6. Arrangement of the vascular tissue at the stem tip. For details see text. In *e* the axillary bud is stippled.

a gap for the incoming trace from the third primordium) and the two branches run alongside the traces from the second pair of primordia. This is illustrated in Text-fig. 7, where the traces are numbered 1, 2, and 3 according to the primordium with which they are associated. In Text-fig. 6*f* the six traces from the third pair of primordia (the large bundles) have entered the vascular ring; they are separated by six composite bundles each of which is made up of a trace from the second primordium and two branches, one from the median and the other from a lateral trace of the first primordium. The branches tend to remain fairly distinct. A foliar trace, either median or lateral, runs through two nodes before it, in its turn, divides. The vascular ring increases in diameter as the stem increases. The vascular strands form

a very close ring, partly because the branches remain distinct (see Text-fig. 6*f*). The cells of the narrow rays of parenchyma between the vascular strands divide tangentially although in the youngest internodes the cells formed remain parenchymatous.



Text-fig. 7. Behaviour of the vascular tissue enclosed in the dotted lines in Text-fig. 6*e*. See text.

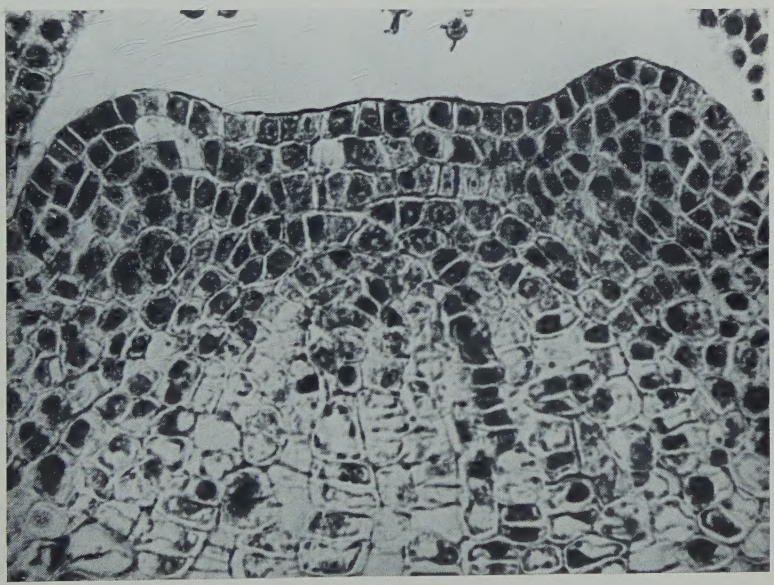
Vascularization of the Axillary Buds

Two vascular traces run to each bud; they come from the branches of the trace which divides to form the leaf gap in connexion with the median trace of the leaf in whose axil the bud is situated. Thus in Text-fig. 7 the bud in the axil of the leaf whose median trace is labelled 3 would receive branches from the traces labelled 1*a* and 1*b*. The branches come off shortly after 1 has divided and before 1*a* and 1*b* are running parallel with their respective traces from the second pair of primordia.

The two strands running to the bud soon subdivide and form a ring of procambial tissue at the base of the bud. When the first pair of primordia on the bud apex are forming, six procambial strands develop from this ring. Further development repeats the vascular organization of the main axis. The vascular connexion to the bud meristem occurs before any primordia are formed.

Acknowledgement

It is a pleasure to record my thanks to my assistant, Miss J. M. Skinner, for preparing all the sections used in this work.



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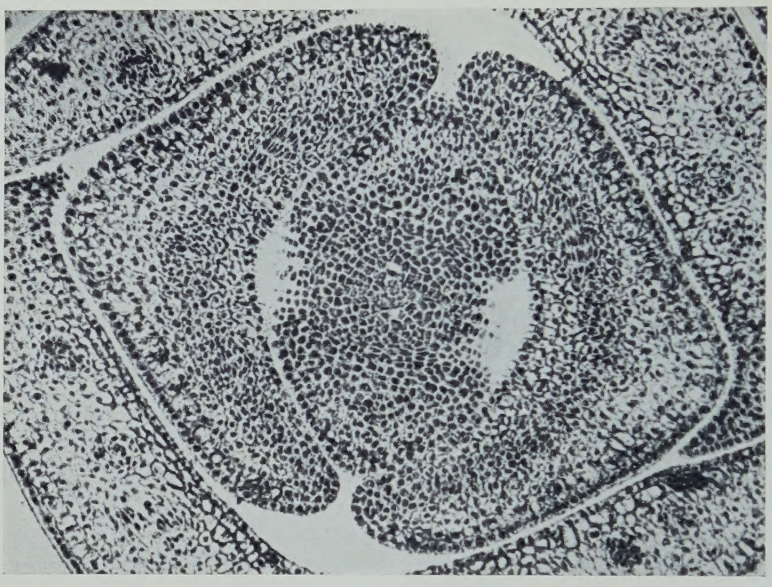

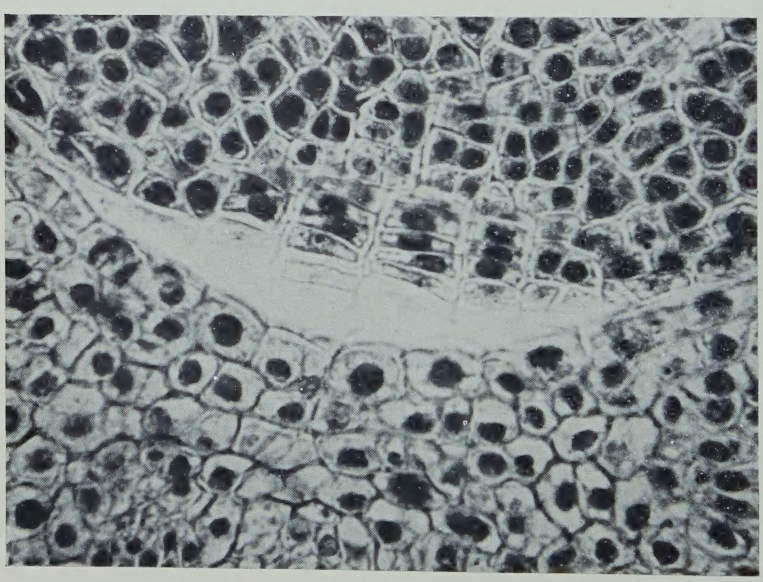

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EXPLANATION OF PLATES

Illustrating D. J. B. White's article on 'The Architecture of the Stem Apex and the Origin and Development of the Axillary Buds in Seedlings of *Acer pseudoplatanus* L.'

PLATE XVI

Apical meristem of sycamore. Top: late phase of plastochron. Below: early phase, with a pair of primordia just forming.

PLATE XVII

Transverse sections of sycamore apex. Top: formation of collar; the cell divisions spreading from base of primordia around flank of apex. Below: an enlarged view of the tissue in which the axillary bud will arise.